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A. WILSKA (HELSINKI) A. I. VIRTANEN (HELSINKI)

EDITOR

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STUDIES ON THE MYOCARDIAL CAPILLARY CONCENTRATION IN CARDIAC HYPERTROPHY DUE TO TRAINING

BY

JUHA HAKKILA

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE WIHURI RESEARCH INSTITUTE, HELSINKI, AND FROM THE INSTITUTE OF FORENSIC MEDICINE, CHIEF PROFESSOR U. UOTILA, UNIVERSITY OF HELSINKI

STUDIES ON THE MYOCARDIAL CAPILLARY CONCENTRATION IN CARDIAC HYPERTROPHY DUE TO TRAINING

AN EXPERIMENTAL STUDY WITH GUINEA PIGS

BY

JUHA HAKKILA

HELSINKI 1955

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MERCATORIN KIRJAPAINO

PREFACE

The present investigation was carried out at the Wihuri Research Institute, Helsinki, under the supervision of its head, Pentti I. Halonen, M.D., Professor of Internal Medicine, University of Helsinki. He suggested the subject of the investigation, placed the laboratory facilities and the experimental animals of the Institute at my disposal, and guided the work through its various phases with never-failing interest, for all of which I extend to him my respectful thanks.

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Helsinki, May 1955

Juha Hakkila

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INTRODUCTION

Since Malpighi in 1663 demonstrated the presence of blood capillaries, these vessels have been a subject of investigation by numerous workers. However, it was not observed until in the nineteenth century that the capillaries do not merely have a passive function in the blood circulation, as was earlier believed (de Haller 1756), but that they are capable of opening and closing when acted upon by various stimulants and thus are able to regulate the blood circulation in the organs (*e.g.*, Philip 1800, Thomson 1813, Hastings 1820, and Wedemeyer 1828).

The extensive study by Ebbecke (1917) contributed further information on the rôle of the capillaries on the vasomotor reactions of the skin and internal organs. Dale & Richards (1918) and Dale & Laidlaw (1919) studied the effect of histamine on the blood circulation and demonstrated the unexpectedly large capacity of the capillary system. A few years later Krogh (1922) published his classical work on the anatomy and physiology of the capillaries, in which he presented observations regarding the capillary blood circulation of the muscles and the metabolic processes that take place between muscle fibres and capillaries. These investigations in the present century added essentially to our knowledge of the structure and function of the capillaries and demonstrated the importance of their rôle in numerous vital functions. At the same time these studies marked a new phase in the research work relating to the capillaries, and interest towards questions in this field began to spread among wider circles of investigators.

The study of the blood capillary system of specifically the heart was started towards the end of the third decade of the present century (Jores 1928 and Wearn 1928). Prior to this, Meigs had in 1899 made attempts to demonstrate the capillaries of the heart by the injection of Berlin blue into the coronary arteries. He did not

succeed in doing so to an adequate degree, but he was able to show that the capillaries in the heart muscle are larger than in other parts of the organism.

Jores (1928) studied the myocardial capillaries in various stages of contraction of the muscle. Following the intravenous injection of trypan blue into the rabbit he stopped the heart at the systole or the diastole by an additional injection of, for instance, potassium sulphide or potassium acetate. Histologic examination of the specimens revealed that the capillaries are wider and more numerous during the diastole than during the systole.

STUDIES ON THE CAPILLARY COUNT IN NORMAL HEART MUSCLE

Wearn (1928) was the first to carry out quantitative studies of the blood capillaries in the heart muscle. In doing so he devised a method of dye injection for demonstrating myocardial capillaries which later has been widely used. He employed twelve normal cat, rabbit and human hearts which had been removed immediately after death. When oxygenated Locke-Rosenheim solution was introduced into the heart in a perfusion apparatus, it recommenced to beat, after which a 2 per cent solution of Berlin blue, or India ink diluted with distilled water in the ratio 1: 1 was injected into the aorta. The myocardial capillaries were well filled with the dye. The capillary concentration [1] was approximately the same in the ventricles, septum and papillary muscles, whereas it was lower in the auricles and Purkinje's system. The ratio was on an average one capillary to one muscle fibre.

Since the publication of Wearn's investigation, three studies have been reported on the capillary count in normal hearts and changes in the number of capillaries during growth (Petrén & Sylvén 1937a, Shipley *et al.* 1937, and Roberts & Wearn 1941). Petrén & Sylvén studied changes in the myocardial capillary concentration in the guinea pig during the foetal stage and during postnatal growth. The capillary counts in their study were based on the erythrocyte staining method developed by Sjöstrand (1934). In

[1] Capillary concentration as used here and later in this report designates the number of capillaries per unit of area measurement.

this method the erythrocytes are stained with benzidine or orthotolidine, and visualisation of the capillaries in the specimens is dependent upon the presence of erythrocytes. To effect a maximum quantitative opening of the capillaries these workers killed the guinea pigs with an intracardiac histamine injection. This investigation showed that the capillary concentration in the heart increased during the foetal period, especially during its last stage, and continued to do so for three weeks after birth. Later during and after the growth period no notable changes occurred in the capillary concentration.

Shipley *et al.* determined the capillary concentration in the heart during its postnatal growth. They also counted the number of muscle fibres in relation to the number of capillaries and measured the diameter of the fibres. The studies were carried out on 24 hearts of growing rabbits and 15 hearts of adult rabbits in which a 2 per cent aqueous solution of Berlin blue was injected into the aorta by the method of Wearn (1928). They found between birth and maturity a seven-fold increase in the gross sectional area of the average fibre, and a nearly six-fold increase in the total length of the fibre. Should new capillaries not have simultaneously been formed, their number in relation to the muscle mass would have been reduced during the growth of the heart. However, the capillary

TABLE 1

DATA FROM STUDIES ON THE MYOCARDIAL CAPILLARY CONCENTRATION DURING FOETAL AND GROWTH PERIODS

Authors	Material Examined	Manner of Determination	Capillaries per sq. mm [1]	
			During Growth	Adult
Wearn 1928 ..	Rabbit	Dye injection	—	5,674
» » ..	Cat	» »	—	4,089
» » ..	Man	» »	—	5,734
Petrén & Sylvén 1937 ..	Guinea pig	Erythrocyte staining	625 (foetus 25 g)	1,965 (guinea pig 625 g)
Shipley *et al.* 1937	Rabbit	Dye injection	3,200 (at birth)	3,420
Roberts & Wearn 1941	Man	» »	3,744	3,343

[1] Capillaries counted in cross section.

concentration remained essentially unchanged throughout the period of growth.

Roberts & Wearn, like Shipley *et al.*, examined the concentration of capillaries and muscle fibres and the diameter of the fibres during and after postnatal growth. Their material was 8 hearts of infants and children and 26 hearts of adults, in which rhythmical contractions were re-established in the perfusion apparatus. The dye injected for demonstration of the capillaries was 2 per cent Berlin blue. These investigators, also, observed that during the process of normal growth of the heart the capillaries multiply at a rate which maintains the capillary concentration in the muscle constant at all times.

The capillary concentrations found in the above mentioned investigations on normal hearts are listed in table 1.

STUDIES ON THE CAPILLARY COUNT IN HYPERTROPHIC HEART MUSCLE

Greatly diverging observations have been reported by investigators who have studied changes in the capillary count in association with hypertrophy of the heart muscle. Thus Thörner (1935), Petrén *et al.* (1936), Vannotti (1936), and Petrén & Sylvén (1937b) found an increase in the capillary concentration as the heart hypertrophied. Their investigations were made in experimental cardiac hypertrophy (guinea pig, rabbit and dog) and the hypertrophy was produced by training with the exception of Vannotti's experiments, in which the hypertrophy was produced either by denervation of the carotid sinus or by producing aortic insufficiency.

Diverging from the above, Shipley *et al.* (1937), Roberts & W arn (1941), Rotta (1943), Linzbach (1947) and Frank (1950) demonstrated a decreased capillary concentration in cardiac hypertrophy, and they were unable to find any increase in the number of capillaries. Only Frank dealt with capillary changes in cardiac hypertrophy produced by training. In the investigation of Shipley *et al.* the hypertrophy was induced by arteriovenous anastomosis, aortic insufficiency or pulmonary stenosis. Roberts & Wearn and Linzbach studied autopsy material, in which the hypertrophy was the

TABLE 2

TABLE 2

DATA FROM STUDIES ON THE MYOCARDIAL CAPILLARY CONCENTRATION IN CARDIAC HYPERTROPHY, PREGNANCY, HYPERTHYROIDISM AND HYPOTHYROIDISM

Author	Material Examined	Age	Cardiac Hypertrophy Produced by	Degree of Hypertrophy [1]	Method of Capillary Determination	Capillaries per sq.mm [2]	
						Controls	Hypertrophy
Thörner 1935	Dog	10—24 mths.	Training	17—41 %	Nuclear staining	—	Increasing
Petrén & Sylvén 1936	Guinea pig	3 »	Pregnancy	No hypertrophy	Erythrocyte staining	1,970	2,160—2,381
Petrén *et al.* 1936 ..	» »	2—134 ds.	Training	43 %	—»—	1,948	2,316
Vannotti 1936	Rabbit	Adult	Hypertension, aortic insuff.	48 %	—»— or dye injection	—	Increasing
Eriksson & Petrén 1937	Guinea pig	2— 4 mths.	Hyperthyroidism	No data	Erythrocyte staining	1,938	2,410
» » » »	» »	1— 2 »	Hypothyroidism	»	»	1,938	1,622
Petrén & Sylvén 1937b	» »	10—29 ds.	Training	64 %	»	1,920	2,961
Shipley *et al.* 1937	Rabbit	1 ½ mths.	Pulmonary stenosis	63 %	Dye injection	3,310	2,740
» » » » ..	»	Adult	Aortic insufficiency, a.-v. fistula, hypertension	54 %	» »	3,420	2,670
Roberts & Wearn 1941	Man	»	Various causes	73 %	» »	3,342	2,483
Rotta 1943	Guinea pig	»	High altitude	≤30 %	» »	3,407	2,964
Linzbach 1947	Man	»	Various causes	Heart wt. > 500 g	Nuclear staining	—	Increasing
» »	»	»	» »	Heart wt. < 500 g	» »	—	Decreasing
Frank 1950	Guinea pig	»	Training	53 %	» »	2,447	1,740

[1] Calculated by the present author from data in the reports, except for the last four investigations.
[2] Capillaries counted in cross section, except by Thörner, who also used longitudinal sections.

result of various pathological conditions. Rotta induced the hypertrophy by keeping the animals in the mountains.

It was also found that the capillaries may increase in number in the hypertrophic heart if it is a question of hypertrophy during growth (Shipley *et al.* 1937) or of certain specified types of hypertrophy in the adult (Linzbach 1947).

A more detailed description is given below of the above mentioned investigations, which differ markedly with regard to material, method and results. The studies in which the capillaries were found to increase in number during cardiac hypertrophy and those in which no evidence was found of the formation of new capillaries are dealt with separately. The principal data on these investigations are listed in table 2.

RESULTS INDICATING FORMATION OF NEW CAPILLARIES

Changes in the nuclear concentration in the hypertrophic dog heart were studied by Thörner (1935) in a small series comprising only two hypertrophic and two control hearts. Cardiac hypertrophy had been induced by having young dogs run daily during several months. From histologic specimens treated with nuclear stain he studied separately the nuclei in the muscle fibres and in the capillary endothelium-connective tissue. The number of nuclei in the muscle as well as in the endothelium-connective tissue was found to be increased during cardiac hypertrophy. In endothelium-connective tissue the increase in the number of nuclei was smaller than in muscle and averaged 12 per cent.

The study made by Petrén *et al.* (1936) originated in the observation made by Petrén & Sjöstrand (1934) that running caused a definite increase in the number of capillaries in the leg muscles of guinea pigs. The test animals used were 47 guinea pigs, mostly 2—3 weeks of age, in which cardiac hypertrophy was produced by running at a speed of 20—67 metres per minute for one-half or one hour daily. The total distance run by the trained guinea pigs was between 24 and 602 kilometres. The controls were 31 non-trained guinea pigs. The animals were killed by an intracardiac injection of histamine and the capillaries were demonstrated by staining the erythrocytes by the method of Sjöstrand (1934). The quantitative determination was based on the assumption that

practically all of the capillaries were filled with blood by the action of histamine. The workers found that the myocardial capillary concentration of the trained guinea pigs increased by an average of 19 per cent as compared with the values for the control guinea pigs. The increase was greatest (45 per cent) in the guinea pigs which had run for the longest time and it occurred more readily in young than in old animals. In the leg muscles the capillaries increased at nearly the same rate as in the heart muscle. In the masseter muscles, on the other hand, no notable change was observed in the capillary concentration.

Vannotti (1936) caused cardiac hypertrophy in adult rabbits either by denervation of the carotid sinus (9 animals) or by producing aortic insuficiency (3 animals). In the former case the capillaries were stained with 1 per cent Berlin blue, using a technique similar to that of Wearn (1928). In the rabbits with aortic insufficiency the capillaries were demonstrated by the erythrocyte staining method of Sjöstrand (1934). In cardiac hypertrophy there was a moderate increase in the capillary concentration. The capillary-fibre ratio, which was in average 1: 1 in the 12 control animals, changed to capillary predominance and was at maximum 2: 1.

Petrén & Sylvén (1937b) observed a definite increase (averaging 50 per cent) in the capillary concentration during cardiac hypertrophy due to the continued effort of running. Their series comprised 46 guinea pigs, 30 of which underwent training. Training of the animals was started at the age of a few weeks and was continued for two to five months. The speed of running was 56—71 metres per minute and the total distance run varied between 150 and 200 kilometres. The animals were treated and the specimens stained as in the study of Petrén *et al.* (1936). Only a part of the trained guinea pigs were killed immediately at the end of the training period, as information was desired on possible changes in the capillary concentration during a period of rest following training. In the animals killed after various lengths of time had passed since training the myocardial capillary concentration was found to have decreased from the level which it had reached immediately after training was stopped. Three months after discontinuance of training the concentration had regained the level of the control series. The connective tissue was not found to be increased when the capillary count reverted to normal.

Shipley *et al.* (1937) demonstrated in connection with the investigation mentioned above (page 9) that the capillary count may increase in cardiac hypertrophy if the latter occurs during growth. This part of their series was small, it is true, comprising only three rabbits in which pulmonary stenosis had been produced at the age of one and a half months. When the animals were killed six months later and the heart capillaries were demonstrated by the method of Wearn (1928), it was observed that the capillary concentration had not decreased as much as they had expected on the basis of fibre hypertrophy. In the opinion of these workers it is difficult to explain this circumstance except by assuming that the tendency of the capillaries to multiply during growth contributed to this result.

An exceptionally high capillary concentration was also seen by Linzbach (1947) in three adult patients with pathological cardiac hypertrophy. He counted the capillaries by the nuclei of their endothelium. In addition to an increased capillary count there also was hyperplasia of the muscle fibres. In these three human cases the cardiac hypertrophy was far advanced. In Linzbach's opinion his investigation demonstrated the existence of a certain borderline (critical heart weight), which is *c.* 500 g for the human heart. At heart weights above this level there may be hyperplasia of the capillaries and muscle fibres of the heart.

RESULTS NOT INDICATING FORMATION OF NEW CAPILLARIES

Shipley *et al.* (1937) found no evidence of the formation of new capillaries in cardiac hypertrophy induced in adult rabbits, contrary to their finding when the hypertrophy was produced during growth. The cardiac hypertrophy was induced by arteriovenous anastomosis (2), hypertension (7) or aortic insufficiency (9). Fifteen rabbits served as controls. The animals were killed five months later and the capillaries were demonstrated by the method of Wearn (1928), using a 2 per cent Berlin blue solution. In addition to the capillary concentration they determined the concentration and average diameter of the muscle fibres. The results obtained showed that the capillary concentration is diminished in hypertrophic hearts in proportion to the hypertrophy of the muscle fibres. According to the investigators, this observation, along with the fact that the ratio of capillaries to fibres did not change, allows a fair conclusion that multiplication of capillaries did not occur.

Using the method employed in the above investigation, Roberts & Wearn (1941) studied the concentrations of capillaries and muscle fibres and the fibre diameters in pathological hypertrophy of human hearts. The part of their investigation relating to changes during growth was already mentioned on page 10. In the part dealing with hypertrophic hearts the series comprised forty adult hearts hypertrophic from various causes, with twenty-six normal hearts of adults as controls. These investigators also found that, proportionate to the degree of hypertrophy of the fibres, there was a lower concentration of capillaries in hypertrophied than in normal hearts. No evidence that the capillaries would multiply in hypertrophy was seen.

Rotta (1943) studied the concentration of capillaries in the heart muscle of guinea pigs in which cardiac hypertrophy had been induced by keeping the animals in the Andes mountains at various altitudes up to 4,500 metres. He used seventy-five guinea pigs of different ages which had been born and raised in the mountain region and fourteen adult guinea pigs born at sea level. The animals were kept for three to six months in the mountains. Twenty-five guinea pigs born and kept at sea level formed the control group. Demonstration of the capillaries was done by the method used by Roberts & Wearn (1941). Rotta found that the capillary concentration decreased in proportion to the degree of cardiac hypertrophy which developed in the mountain region. Enlargement of the muscle fibres was most distinct at high altitudes and was proportionate to the decrease in the capillary concentration.

Frank (1950) studied the myocardial capillaries in guinea pigs with cardiac hypertrophy due to swimming. Eighteen animals weighing 500—600 g were made to swim daily during four months, at first for short periods but finally up to two hours at a time. Nine of the trained animals died before the end of the training period. The control group consisted of nine non-trained guinea pigs. For the demonstration of the capillaries, histamine was injected intracardially under anaesthesia, followed by the injection of 20 per cent India ink. As in the preceding three investigations, a definite decrease in the capillary concentration was seen in the hypertrophic hearts. On comparison of the control and hypertrophic hearts he was unable to find any signs of multiplication of the fibres or any change in the relationship between fibre and capil-

lary numbers. Regardless of advanced myocardial hypertrophy (average 53 per cent), the electrocardiographic and histological examinations revealed no evidence of myocardial degeneration.

STUDIES ON THE CAPILLARY COUNT IN HEART MUSCLE DURING PREGNANCY, HYPERTHYROIDISM AND HYPOTHYROIDISM

Petrén & Sylvén (1936) studied changes in the capillary count in the guinea pig heart due to pregnancy. The study was made on thirty-five pregnant and fifteen control animals and the erythrocyte staining method of Sjöstrand (1934) was used for demonstration of the capillaries.Although actual cardiac hypertrophy was not found, the capillary concentration in the hearts of the pregnant guinea pigs was on an average 10 per cent higher than in the control animals. The greatest increase (20 per cent) was seen towards the end of gestation.

Eriksson & Petrén (1937) produced thyreotoxicosis in ten guinea pigs by the intra-abdominal injection of thyroxin and found, on an average, a 25 per cent[1] increase in the capillary concentration, as compared to nineteen healthy guinea pigs used as controls (erythrocyte staining of Sjöstrand). In five thyroidectomised guinea pigs there was a decrease (up to 25 per cent[1]) in the capillary concentration. The presence of possible hypertrophy is not mentioned in their report.

[1] Percentage calculated by the present author.

OBJECT OF THE INVESTIGATION

Investigations on the myocardial blood capillaries are an important field of study in the physiology and pathology of the heart.

As was seen in the preceding review of the literature, the findings made and conclusions drawn have been greatly contradictory in the few investigations in which quantitative determinations of the capillaries have been carried out in hypertrophic hearts. Since the capillary determinations in each type of hypertrophy were not made by more than one method in each investigation, it may be possible to ascribe the divergent results to marked differences in the methods used. They may also have been caused by differences in the methods of production or the etiology of cardiac hypertrophy, as well as by dissimilarities in the material studied by different investigators. For these reasons the present author considered it desirable to study the behaviour of myocardial capillaries in cardiac hypertrophy.

This study was made on young guinea pigs, in which hypertrophy of the heart was produced by training. Quantitative determinations of the capillaries were made by different methods, taking especially into consideration at the same time the possible sources of error inherent in the various methods employed.

It was the object of this investigation to obtain an answer to the question whether or not an increase occurs in the number of capillaries during experimental cardiac hypertrophy produced by training.

PRESENT INVESTIGATION

STATISTICAL TREATMENT

The following statistical treatment was employed to determine the significance of the results obtained in the investigation.

The standard error s_x of a single observed value x_i is obtained from the following formula:

$$s_x = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

where $\bar{x}$ is the arithmetical mean of the series of observed values and n represents the number of observed values. The standard error of the arithmetical mean $s_{\bar{x}}$ is obtained from the formula:

$$s_{\bar{x}} = \frac{s_x}{\sqrt{n}}$$

The result from the series of observed values is presented in the following form:

$$\bar{x} \pm s_{\bar{x}}$$

To find out the significance of the difference between the arithmetical means $\bar{x}$ and $\bar{y}$ of two series of observed values, Student's t-test was applied, as follows:

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{n+m}{nm} \cdot \frac{(n-1)\, s_x^2 + (m-1)\, s_y^2}{n+m-2}}}$$

where n and m indicate the number of observed values in the two series and s_x and s_y the standard error of a single observed value in each series.

The interdependence of two series formed of pairs of observed values was determined by calculating the correlation coefficient r from the following formula:

$$r = \frac{\Sigma(x_i - \bar{x})\ (y_i - \bar{y})}{(n-1) \cdot s_x \cdot s_y}$$

where x_i and y_i are the individual observed values in the series, $\bar{x}$ and $\bar{y}$ the arithmetical means of the series formed of pairs of observed values, and s_x and s_y the standard errors of the respective values.

Student's t-test was applied for determination of the significance of the correlation coefficient r calculating t from the formula:

$$t = \sqrt{n-2} \cdot \frac{r}{\sqrt{1-r^2}}$$

The dependence of observation y from its observation pair x in a series of observation pairs is shown by the regression line, which is obtained from the following formula:

$$y = \bar{y} + \frac{s_y}{s_x} \cdot r \cdot (x - x)$$

The significance of the t-value is obtained from the t-table. A value with a probability of 95.0—99.0 per cent is concidered almost significant, with a probability of 99.0—99.9 per cent significant, and highly significant if it is higher than 99.9 per cent.

PRELIMINARY STUDIES

Since the author's previous personal experience in matters relating to the study of myocardial blood capillaries was not adequate, a number of experiments were carried out for the selection of the methods most suitable for the main investigation. The possible influence on the results of certain sources of error were studied at the same time.

The guinea pig was chosen as the test animal for the preliminary experiments. This animal had been used by the earlier workers, with the exception of Thörner (1935), for studies on blood capillaries in hearts hypertrophied by training (Petrén *et al.* 1936, Petrén

& Sylvén 1937b, and Frank 1950). A total of 97 guinea pigs were used in the preliminary tests.

Swimming as a means of producing cardiac hypertrophy was abandoned after short testing, for several of the guinea pigs died already in an early stage of training by swimming, as had also occurred in Frank's experiments. It was therefore considered preferable to train the guinea pigs by running. With this method only very few animals were unable to complete the training. By making the animals run on a moving belt it was possible to regulate the intensity of the training and to maintain it at the same level for all the animals, as the entire group could be handled at the same time.

To determine the appropriate training intensity for the main investigation, a group of guinea pigs was trained and the capillaries were examined by the erythrocyte staining method of Sjöstrand (1934). The results obtained in this group were not satisfactory in all respects and different procedures were therefore tested in order to avoid the sources of error. For this purpose the hearts were frozen by liquid air, using the method devised by Sjöstrand (1937). The suitability of a combined erythrocyte and nuclear staining for the capillary determinations was also tried out, and methods of injecting various dyes for the demonstration of myocardial capillaries according to the method of Wearn (1928) were tested.

These preliminary experiments are described below. However, numerous details are omitted here, being described more closely later in this report in connection with the presentation of the material and methods of the main investigation.

TRAINING TESTS

For the preliminary tests, the training of eight guinea pigs by running was started at the age of three weeks, when the animals had a mean body weight of 179 g. To obtain some idea of the suitability of also older guinea pigs for this purpose, another group of eight animals weighing on an average 512 g was trained. Eight controls were used in both series. Only male guinea pigs were used.

The animals were made to run on a wide belt operated by an electric motor (fig. 1, page 29). At first the guinea pigs ran slowly, but as they became trained the length of daily training was extended

and the speed was increased up to 45 metres per minute. Three weeks after commencement of the training the guinea pigs were able to run at this speed for one-half hour once daily, or a distance of 1,350 metres. Training was then continued unchanged during the remainder of the training period. During the three months of training the guinea pigs had run about 100 kilometres.

Of the young animals, one had to be eliminated because of a gradually impaired running power and one because of leg injury. The six young animals which completed the training showed no signs of fatigue during training. The older guinea pigs frequently ran unevenly but even they showed no fatigue. When training was finished the animals were killed by an intracardiac injection, under ether anaesthesia, of 1 mg of histamine phosphate (British Drug House, Poole) per kilogram of body weight. When the animal died 3—6 minutes after the histamine injection, the thorax was opened and the heart was dissected out immediately after beating had ceased. After removal of the pericardium and the auricles and emptying of the blood from the ventricles, the hearts were weighed and treated according to the erythrocyte staining method of Sjöstrand (1934), as follows: The specimens were fixed in formalin diluted with water 1: 5, and after being rinsed in water for 24 hours were treated with acetone and benzene and embedded in paraffin. The hearts were cut into two halves (fig. 2, page 33) and slices were cut at 15 μ and 5 μ from the median surface of each half. The slices 15 μ thick were stained with benzidine to demonstrate the erythrocytes, and the slices at 5 μ were stained with Delafield's haematoxylin for the demonstration of nuclei.

Only the capillary concentration was determined in the preliminary experiments. This was done from the erythrocyte-stained specimens with the aid of a cross-ruled ocular micrometre. All counts were made in 405-fold magnification from fields in which the muscle fibres were cut in perfect cross section and the distribution of capillaries was as even as possible. Such areas were usually small and far apart, which rendered it difficult to make a sufficiently large number of observations. Some disturbance in the counting procedure was also caused by shrinkage during the histologic treatment, manifested as islets of tissue formed by clefts. According to the nature of the specimens, the number of capillaries counted in each heart varied from *c.* 500 to 1,000.

RESULTS OF TRAINING TESTS

Macroscopic examination of the killed animals revealed no appreciable difference between the young trained guinea pigs and the control animals. In the older trained animals the fatty tissue was diminished in amount in comparison to the respective control guinea pigs.

Table 3 shows the mean body and heart weights and the mean myocardial capillary concentrations of control and trained animals. In order to give a better picture of the effect of training on the body weight, the increase in the body weight of each animal during the training period was determined. However, no statistically important difference was observed when the mean increases in the control and trained groups were compared. The mean increase for young controls was 223 ± 6 grammes and for young trained animals 228 ± 15 grammes, the difference being $+5 \pm 15$ grammes ($t = 0.34$). For the older animals the corresponding mean values were 154 ± 12 grammes and 116 ± 15 grammes and the difference -38 ± 19 grammes ($t = 2.00$).

No statistically definite myocardial hypertrophy was demonstrable on the basis of the heart weights in the two groups of animals, the difference in the young guinea pig group being $+75 \pm 74$ mg ($t = 1.01$) and in the older group $+62 \pm 97$ mg ($t = 0.64$). The capillary concentrations, also, showed no noteworthy differences between the controls and the trained animals. For the young animals the difference was $+35 \pm 240$ capillaries per square millimetre ($t = 0.14$) and for the older animals $+119 \pm 116$ capillaries ($t = 1.03$).

The number of observations made was limited because of an irregular occurrence of capillaries and the sparseness and relatively

TABLE 3

MEAN BODY AND HEART WEIGHTS AND MYOCARDIAL CAPILLARY CONCENTRATIONS OF CONTROL AND TRAINED GUINEA PIGS IN PRELIMINARY TESTS

Experimental Group	No. of Animals	Body Weight (g)		Heart Weight (mg)	Capillaries per sq. mm
		Initial	Final		
Young controls ..	8	179	402	1,515±42	3,675±164
Young runners ..	6	179	407	1,590±66	3,710±168
Older controls ..	8	512	666	2,239±71	3,092± 84
Older runners ..	8	520	636	2,301±66	3,211± 79

small size of satisfactory counting fields in the examined slices. This may account for the fairly great variations in the mean capillary concentrations in the different hearts. For this reason the results obtained probably cannot be considered fully reliable.

Specimens in which nuclear staining had been carried out in addition to the erythrocyte staining showed stained capillary endothelial nuclei also in areas in which the erythrocyte staining demonstrated no red cells, indicating the presence of empty capillaries in these areas.

From these observations the conclusion was drawn that erythrocyte staining in the manner tested by the author was not suitable for quantitative determinations of blood capillaries.

EXPERIMENTS CONNECTED WITH THE FREEZING METHOD

In working out his method for the demonstration of myocardial blood capillaries by the injection of dye into the perfused heart Wearn (1928) observed unsatisfactory filling of the capillaries when the heart was allowed to gradually cease beating after the injection. He suggested that the heart muscle »milked» the dye from the capillaries, mainly through the veins of Thebesius. Since a similar phenomenon probably may have occurred during the last stage of heart beating in the experiments described above, tests were made to interrupt the heart function more rapidly by freezing the heart with liquid air, as was done by Engstrand (1942).

A number of tests were performed for the selection of suitable procedures in connection with the freezing method. In these tests the thorax of the anaesthetised animal was opened and a lethal injection of histamine was given into the left ventricle. Around the heart was then placed a paper funnel, into which liquid air was poured from a thermos bottle.

After testing various freezing periods, 20 seconds was considered a suitable length of time. If it exceeded 25 seconds, clefts which were troublesome in the later manipulation of the specimen were liable to occur in the heart.

Since equipment was not available for vacuum evaporation, the frozen hearts were fixed by a method developed by Sjöstrand (1937). Immediately after freezing the hearts were chiseled out and placed at once in formalin in a refrigerator freezing compartment

with an average temperature of —8° C. At this temperature the fixation of the specimens took place gradually without the tissue destruction or blood cell haemolysis which usually attend defreezing. Since it was necessary to use formalin diluted 1: 1 to prevent its freezing at the fixation temperature, alterations occurred in the finer details of the tissues; this sometimes caused difficulties in the determination of muscle fibre margins or counting of capillaries by the endothelium and its nuclei. Attempts were therefore made to employ for fixation also various aqueous dilutions of alcohol or alcoholic solutions of formalin. However, since they had an adverse effect on the stainability of red cells, fixation in the formalin was resumed. A period of 10 days was found to be the most suitable fixation time. After shorter periods the centre of the heart still remained frozen.

In experiments to find the best time when freezing should be started, early freezing was first attempted. The hearts were frozen 10 seconds after the injection of histamine. Electrocardiograms were taken of some guinea pigs during the freezing procedure. Those ECG's which turned out satisfactorily showed an unchanged heart rate and PQ interval regardless of the freezing — an observation also made by Sjöstrand (1937). The electrocardiographic signs of heart function disappeared 8 to 10 seconds after the freezing procedure was started.

Examination of the erythrocyte-stained specimens from these hearts revealed that the capillaries were visible only in some places, and it was difficult to find areas in which the capillaries were evenly filled with red cells. To obtain some opinion regarding the capillary concentration in these hearts, the capillaries were counted in fields showing the largest number of capillaries. The concentration was found to range in different hearts from *c.* 2,800 to 3,200 per square millimetre.

Later freezing times gave definitely better results. Thus, for instance, hearts frozen 4 minutes after the histamine injection was made, less frequently had areas in which the capillaries were not brought out by erythrocyte staining. However, even in these specimens the capillaries were quite evenly demonstrated in exceptional cases only. The capillary concentration varied in the different hearts from *c.* 3,400 to 3,900 per square millimetre. When freezing was carried out 8 minutes after the histamine injection, the heart func-

tion was clearly weaker than 4 minutes earlier and the rhythm was irregular. The electrocardiographic signs of heart function disappeared already 4 to 6 seconds after freezing was started. In nearly all of the examined specimens the capillaries were considerably more evenly filled with blood than in the preceding specimens. In fields in which the capillaries were cut in perfect cross section they usually were evenly spaced. In such specimens the capillary concentrations ranged from *c.* 3 700 to 4,100 per square millimetre. This freezing time made it possible to obtain a relatively even distribution of stained capillaries and to count the capillaries from numerous fields in each heart. It was therefore chosen for use in the main experiments in this investigation.

Since the erythrocyte staining method brings out only the erythrocytes and leaves other tissue elements nearly unstained, these specimens could not be used for quantitative determinations of muscle fibres, which often are of significance in capillary studies, for instance in the interpretation of possible changes in the number of capillaries by determination of the fibre-capillary ratio. To colour the muscular tissue, after-staining with eosin was therefore used in combination with the erythrocyte staining. However, since staining with eosin alone was also able to give to erythrocytes a sufficiently intense colour to make quantitative studies possible, it was decided to carry out parallel capillary determinations not only from specimens stained by Sjöstrand's method for erythrocytes but also from specimens stained with eosin. As the nuclei in the latter specimens were stained by the haematoxylin staining method of Delafield, the capillaries could be counted also from the endothelial nuclei even in the cases in which the capillaries did not contain eosin-stained erythrocytes. The staining of also the capillary endothelium by haematoxylin-eosin was a further aid in the counting of capillaries.

In connection with these preliminary tests, certain control experiments were also made to determine the significance of shock as a possible factor influencing the capillary determinations based on erythrocyte staining. This was considered necessary since it seemed possible that differences in resistance to histamine shock might influence the blood content of the capillaries. In this connection may be mentioned the investigation by Kjellberg *et al.* (1949a) demonstrating that training may greatly increase the blood volume. In the training of guinea pigs, a factor of this kind may give

the trained animals a greater endurance against histamine-induced shock than the control animals possess, and the myocardial capillaries of the trained animals may therefore contain more blood than those of control animals (Sjöstrand 1952).

To study the shock factor the hearts of eight guinea pigs of about 600 g body weight were frozen 4 minutes after the intracardiac injection of histamine. At this stage the histamine shock was already far advanced in the animals, as judged by the weak pulsation of the aorta on palpation. In another group of eight guinea pigs of about the same weight the aorta was compressed by clamps 10 seconds after the histamine injection. The hearts were frozen 4 minutes later in the same manner as in the preceding group. Because of closure of the aorta its pulsation at the moment of freezing was still distinct and the heart function was also definitely stronger than in the guinea pigs in the preceding group.

The animals subjected only to freezing of the heart after the histamine injection had a mean capillary concentration of $3{,}663 \pm 42$ per square millimetre. In the guinea pigs with compressed aorta it was $3{,}441 \pm 32$ per square millimetre. The difference between the above mentioned capillary concentrations (-222 ± 55) is statistically highly significant ($t = 4.01$). However, in evaluating the significance of this result it is to be taken into consideration that, on the other hand, the definitely stronger heart action of the guinea pigs with compressed aorta may be a factor influencing the results.

EXPERIMENTS CONNECTED WITH THE DYE INJECTION METHOD

A third method tested in the preliminary experiments consisted of the injection of a dye for demonstration of the blood capillaries. The perfusion method devised by Wearn (1928) seemed to be most suitable for this purpose. An apparatus of the Langendorff type was constructed in which contractions of the isolated hearts were reestablished by perfusion of the organ with warm, oxygenated perfusion solution.

Under the usual ether anaesthesia, the guinea pig's thorax was opened, the aorta compressed with a clamp and the heart removed. A cannula was introduced into the aorta and the heart was transferred to the perfusion apparatus.

Of the various perfusion solutions tested — Ringer's solution

(for warm-blooded animals), Langendorff's heart perfusion solution, and Locke's solution [1] — the last mentioned was found to be best for this purpose. With this solution the hearts contracted rhythmically for longer periods than with the other solutions. Since, according to Engstrand (1942), the capillary concentration and the capillary blood volume in the heart increase with an increased heart rate, +39° C was selected as the temperature of the perfusion fluid. The rate and force of heart beats was greater at this temperature than when the perfusion fluid was of the usual temperature of +37°C. The perfusion pressure was 50 mm of mercury, as previously used by Rotta (1943).

The suitability of various dyes for demonstration of the capillaries by the injection method was then tested. After injection of 2 per cent Berlin blue the capillaries were well visible, but, as already observed by Shipley *et al.* (1937), the dye did not remain long in the capillaries but tended to become diffused in the surrounding tissue, thus making examination difficult if not carried out soon after the specimens were sectioned.

In view of these drawbacks in the use of Berlin blue, two graphite suspensions were tested (EB 1085 and EB 1015, Acheson Colloid, London). The former contained 22 per cent colloidal graphite suspended in physiological saline and the latter 1.04 per cent in aqueous suspension. The capillaries in the specimens were incompletely filled with the first mentioned suspension, possibly due in part to the large size of the graphite particles, which according to the manufacturer's statement may in a small percentage be as large as 10—12 μ. Better results were obtained with the latter suspension, in which only 5 per cent of the particles were over 1 μ but less than 4 μ. However, although the capillaries were evenly filled, counting was difficult because of poor visibility owing to the low graphite concentration.

Tests were also made with India ink as the injection medium. Of the solutions available, the choice was a 5 per cent aqueous suspension of India ink (Indian Ink Micro, George T. Gurr, London), which according to the manufacturer's statement contained particles over 0.5 μ but less than 2 μ in 5 per cent only. The capillaries were clearly visible and their distribution was very even in the hearts

[1] Locke's solution was prepared as follows: NaCl 9.0; KCl 0.2; $CaCl_2$ 0.2; $NaHCO_3$ 0.2; dextrose 1.0; distilled water to make 1,000.0.

injected with this suspension. In slices showing capillaries cut in cross section they were spaced at similar regular intervals as in the erythrocyte-stained specimens when the latest time of freezing was used.

Hearts injected with India ink were treated by the gelatin method of Heringa & ten Berge and slices were cut with a freezing microtome for calculation of the capillary concentration. These specimens showed a concentration of nearly 2,500 capillaries per square millimetre.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Using the experiences gained in the preliminary studies, training experiments were carried out with 80 male guinea pigs divided into two groups. Each group comprised 20 guinea pigs trained by running and 20 untrained controls. The animals were three weeks old when training was started.

All the guinea pigs were procured from the same breeding station and were healthy animals of the same strain and uniform parentage. They were taken from litters of not more than 4 days' difference in age. The litters were separated from their mothers two days before commencement of the experiments.

During the experimental period the guinea pigs were kept in roomy cages, each containing ten animals. The temperature of the stable varied from $+17°$ to $+21°$C. The animals had free access to fresh or dried hay, according to the season, and to Swedish turnip and smaller amounts of cabbage, red beets and oats. In winter, vitamin C in the amount of 1 mg per kilogram of body weight was mixed with the oats once weekly.

The guinea pigs were weighed before commencement of the training experiment and immediately before being killed. Weighing was carried out at identical times in the morning before the animals were fed.

The heart rate was determined by auscultation at rest and the mean rate was calculated from several determinations made at the time of weighing before the morning training.

The physical condition of the animals was observed closely throughout the experimental period. Sick animals were discarded. Thus three trained animals and three controls were eliminated during training due to illness, as follows: From group I, one trained guinea pig because of abscess due to biting, and two control guinea pigs because of, respectively, infection of the eye and inflammation of cervical nodes; from group II, one trained guinea pig because of an injured leg, and one trained guinea pig and one control animal because of pneumonia. Three guinea pigs were also discarded from group II because of disinclination to run.

After fasting overnight, the animals were killed in the morning following the last training day. One animal from the training group and one from the control group were killed alternately.

TRAINING

The animals were made to run on a belt operated by an electric motor. The apparatus constructed for this purpose was similar in type to that designed by Petrén (1934). It consisted of rubber matting 0.8 m wide, spliced end to end and revolving over two rollers 1.2 m apart. The speed of the belt was regulated by means of a set of pulleys attached to one of the rollers. To induce the animals to run, revolving cylindrical brushes were attached to the apparatus behind the animals. The belt accommodated twenty guinea pigs separated by partitions (fig. 1).

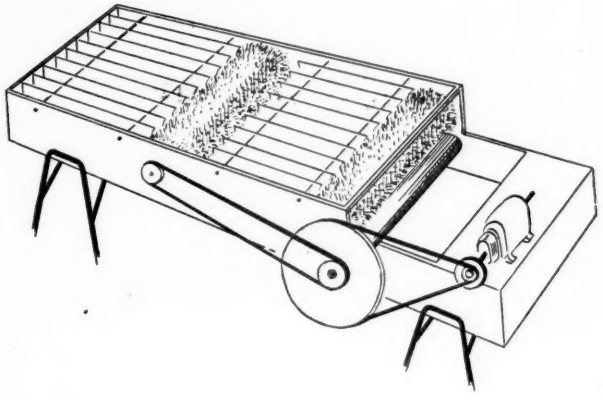

Fig. 1. — Electrically operated apparatus for training of the guinea pigs.

During training, special attention was paid to each animal's individual capacity for running and to changes in this capacity. The usual signs of excessive strain were an uneven pace and an exceptionally long period of laboured breathing after training. On the occurrence of signs pointing to overstrain the training time was reduced to one-half for two to four days, after which the animals usually were able to run as well as earlier. Group I was trained in the autumn of 1952 and group II in the spring of 1953. The training was carried out daily at 10 a.m. and 8 p.m. and was continued for 3½ months. It was attended to by the author.

In *group I* the training program was similar for all the guinea pigs irrespective of their individual running capacity. It was started by training the animals only once daily. The speed at this time was 20 metres per minute and the training time 5 minutes. The time was gradually extended to 30 minutes and the speed was increased up to the rate of 60 metres per minute. Three weeks after the commencement of training all the animals in this group were able to endure this strain. It was then increased by having the animals run also in the evening. One month later their endurance had increased to the point where they were able to run twice a day for 30 minutes at a time at the speed of 60 metres per minute, or a distance of 3.6 kilometres per day. The training was continued at this rate for seven weeks. The average total distance run by this group was 260 km.

In *group II* it was the object to submit all the guinea pigs to the maximum endurable effort. For this reason the training differed from that in group I by being further increased during the last seven weeks according to the individual capacity of the animals. As far as possible, the running period was of equal length in the morning and evening. On the last days of training the distance run daily by the best guinea pigs was 7.2—10.8 km, which is equal to 2—3 hours' running. The daily performance of the poorest runners was of the same order as of the animals in group I. The best guinea pigs ran total distances of 340—410 km and the poorest 280 km. When the training time was extended to over one hour daily, three of the animals in this group were unable to follow even when the strain was eased, and they ultimately showed a complete disinclination to run. They were therefore discarded from the experiment.

ELECTROCARDIOGRAPHIC STUDIES

Electrocardiograms were made of the guinea pigs in group I at the age of 3 weeks, before training was started, and immediately before the animals were killed. In group II they were made only before killing. All the electrocardiograms were done under ether anaesthesia, using the usual extremity leads with needle electrodes. If not anaesthetised, the animals would have disturbed the results by their restlessness. The apparatus used in these studies was either a string galvanometre electrocardiograph (Siemens, Germany) or a directly recording Mingograph (Elema, Sweden). The electrocardiographic studies of the guinea pig described by Frank (1950) and Luisada (1953) were used as an aid in the interpretation of the electrocardiograms.

PREPARATORY MEASURES IN THE TREATMENT OF THE MATERIAL

In *group I* the guinea pigs were tracheotomised under ether anaesthesia, which then was maintained through a tracheal cannula. An anaesthesia apparatus was constructed specifically for this purpose along the principle of those used in anaesthesia with artificial respiration. An ordinary Record syringe was employed as pump. During the anaesthesia the concentration of the ether-oxygen mixture and the pressure employed could be adjusted according to requirement.

The thorax of the animal was so opened that a portion of its anterior wall could be lifted up. An injection of 1 mg of histamine phosphate (British Drug House, Poole) per kilogram of body weight was injected into the left ventricle. Eight minutes later the heart was frozen with liquid air. To promote rapid freezing it was slightly elevated, supported by the membranes attached to the sternum, and a funnel of blotting paper was inserted into the thorax so that the heart lay loosely inside the funnel. Liquid air was then poured into the funnel from a thermos bottle for 20 seconds, during which the heart was kept covered with the intensely boiling liquid. The frozen hearts were chiseled out and immediately transferred for fixation into a freezing compartment in the refrigerator, where they were kept at a temperature of, on an average, —8° C.

In *group II* the thorax was opened under the usual ether anaes-

thesia, the aortic arch was compressed with a clamp, and the blood vessels arising from the heart were carefully severed. The heart was then transferred to Locke's solution, under which the aorta was exposed and severed below the base of the carotid arteries. A short polythene cannula with a rough external surface was introduced into the aorta to a distance well above the aortic valves. The aorta was fastened tightly around the cannula with a loop of silk.

Taking care that no air should enter the cannula, the heart was then placed in the Langendorff type perfusion apparatus and contractions were re-established by perfusion of the heart with warm oxygenated Locke's solution. A fresh batch of this solution was prepared immediately before use. The solution was saturated with oxygen, and oxygenation was continued during perfusion. The oxygen was passed through a dense filter to remove the dust. The perfusion solution was heated to +39° C in a thermostat, where excess oxygen escaped. The perfusion pressure of 50 mm of mercury was maintained by means of the hydrostatic pressure of the Locke's solution in a Mariotte flask.

The heart, which had ceased to beat, recommenced its contractions a few seconds after perfusion was started. When a rhythmic rate had been established and the perfusion fluid flowed evenly from the heart, a 5 per cent solution of India ink (Indian Ink Micro, George T. Gurr, London), temperature +39° C, was injected for demonstration of the capillaries. With a 10 ccm syringe attached to the apparatus this solution was injected into the space superior to the point of insertion of the cannula into the apparatus, whence the solution passed into the heart through pressure of the perfusion solution. Immediately after injection the heart was stained coal black. Into the aortic cannula was then injected 0.5 ccm of glacial acetic acid, which immediately stopped the beating of the heart. The heart was then removed from the apparatus, and the pericardium and auricles were removed, the fluid was blotted from the ventricles, and the heart was weighed and placed in formalin diluted 1: 10.

HISTOLOGICAL TECHNIQUE

The frozen hearts of the guinea pigs in *group I* were placed for fixation in formalin diluted 1: 1 for 10 days at —8° C. The peri-

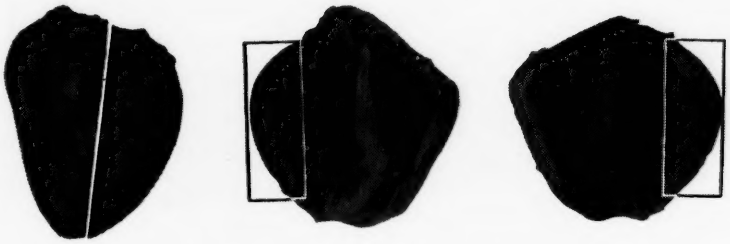

Fig. 2. — Photograph showing manner of cutting guinea pig hearts into two parts after fixation (left) and area from which all determinations were made (centre and right).

cardium and auricles were removed and the heart was cut in the frontal plane into two sections of equal size (fig. 2) and the sections were placed in running water for 24 hours.

The hearts were then blotted and weighed and the blood was removed mechanically from the ventricles as completely as possible, taking care not to injure the tissues. The hearts were then reweighed. In accordance with Sjöstrand's erythrocyte staining method (1934) the specimens were passed through acetone into benzene, in each of which they were kept for 4 hours. The paraffin used had a melting point of +54° C, and the same paraffin was used for embedding of the specimens. Slices were cut from both halves of the heart at 15 μ and 5 μ parallel to the sectioned surface. In addition to slices from the surface, other slices of the same thicknesses were cut at four deeper levels at intervals of 300 μ. The slices at 15 μ were stained by Sjöstrand's erythrocyte staining method. For better contrast, orthotolidin solution of the same concentration was substituted for the benzidine, as recommended by Sjöstrand in describing his erythrocyte staining method. The slices at 5 μ were stained with Delafield's haematoxylin and after-stained with eosin.

In *group II* the hearts were kept for two days in 1: 10 diluted formalin. After rinsing under running water for 24 hours, the hearts were cut into two halves as in group I. They were then treated by the gelatin method of Heringa & ten Berge, according to which they were kept for 24 hours in a 10 per cent aqueous solution of gelatin at +37° C and for a further 24 hours in a 20 per cent solution at the same temperature. The specimens were embedded in the latter solution. Using the freezing microtome 15 μ slices were cut from the

two halves at four levels 300 μ apart. After cutting, the slices were placed in water to which eosin had been added for staining of the muscular tissue. The specimens were removed from the water direct to the object glass, covered with a cover glass and examined while still wet.

EXAMINATION OF HISTOLOGICAL SPECIMENS

The same microscope (Cook, York) was used in all the histological examinations. All counts were made from the middle muscular layer of the left ventricle (cf. fig. 2), where the muscle fibres usually were cross-sectioned.

A magnification of 90 times was used to find suitable fields for counting. A 405-fold magnification was selected for the counting operation. The blood capillaries were counted only in areas where they were cut in cross section, selecting those fields in which the distribution of capillaries was of maximum evenness and avoiding fields whose continuity was broken by clefts or large veins or arteries. All blood vessels above the capillary size that was usual in these specimens were disregarded in the count.

In specimens stained by Sjöstrand's erythrocyte staining procedure the count was based on the staining of red cells in the capillaries. In the specimens stained by Delafield's haematoxylin-eosin the capillaries were counted not only by means of the red cells within the capillaries but also by the capillary endothelium and its nuclei. In the specimens stained by injection of India ink the capillary count was made on the basis of their India ink content.

For counting of the capillary concentration, a square, cross-ruled micrometre divided into 25 squares of equal size was fitted into the ocular. In 405-fold magnification the cross-ruled area bounded a field of 46,225 square micra, with sides 215 μ in length. The microprojector used by Uotila & Kannas (1952) in their histological studies was also employed as an aid. The specimen was projected on paper by means of this appliance in 480-fold magnification, and the boundaries of suitable fields and the position of the capillaries within this field were drawn on the paper. The number of capillaries in the field was then counted and the area of the field was determined with a planimetre (Corad, Zürich).

At the same time as the capillary concentration was calculated,

the diameter of the muscle fibres and the fibre-capillary ratio were also determined in areas in which the fibres were sufficiently clearly demarcated. The fibre diameters were determined with an ocular micrometre from the fibres which touched a single point of the micrometre as the specimen was moved laterally. The maximum and minimum diameters were measured and their mean was regarded as the diameter. Twenty fibres were measured in this way in each of the fields from which counts were made.

The calculations from the histological specimens in the two groups of guinea pigs were made as follows.

In *group I*, parallel calculations were made of the capillary concentration in the specimens stained by Sjöstrand's erythrocyte and Delafield's haematoxylin-eosin staining methods. In addition, the muscle fibre-capillary ratio and the mean diameter of cross-sectioned fibres were determined for each heart in this group from the specimens stained with haematoxylin-eosin, which differentiated the details of tissue structure more clearly than when erythrocyte staining alone was used.

To eliminate drawbacks due to shrinkage of the specimens, the capillaries in the erythrocyte-stained specimens were counted by means of the projection and planimetric measurement method. This method had the important advantage that the capillary concentrations could be counted from larger areas without being disturbed by clefts in the tissue. About 3,000 to 5,000 capillaries were counted from each heart stained for erythrocytes.

The projection method was not suitable for the haematoxylin-eosin stained specimens in view of their poorer transparency. The capillary concentrations were therefore determined by means of the cross-ruled ocular micrometre, usually over an area of 2—10 squares. About 1,500 to 2,000 capillaries were determined from individual hearts.

To determine the fibre-capillary ratio, about 500—1,500 muscle fibres were counted from each heart. The mean diameters of muscle fibres were determined for c. 100—300 fibres.

In *group II* the capillaries were counted with the aid of the cross-ruled ocular micrometre. Because of the smaller degree of shrinkage, these specimens did not have clefts which would have prevented the counting of large continuous areas and it was possible in all cases to determine the capillary concentration over the

whole cross-ruled field. The number of capillaries counted ranged from about 3,000 to 4,000 per heart. About 1,000 muscle fibres were counted in each heart to determine the fibre-capillary ratio. The mean diameter of fibres was calculated from measurements of about 400 fibres in each heart.

CRITIQUE OF THE METHODS

Owing to the nature of the methods employed, several possibilities of error are inherent in the quantitative determinations of myocardial capillaries. The elimination of these errors to the maximum degree possible is a condition for the procurement of reliable results. Certain circumstances which are associated with the methods of investigation employed and which may influence the numerical determination of the capillaries or the interpretation of the observations are discussed below in detail, each method being dealt with separately for the sake of clarity. Individual consideration is also given to the histological technique and examination of the specimens. Certain factors that possibly should be taken into consideration in the evaluation of the results are also mentioned.

SOURCES OF ERROR IN THE ERYTHROCYTE STAINING METHOD

Criticisms have been expressed by some investigators (Campbell 1938, Roberts & Wearn 1941, Linzbach 1947 and de Langen 1953) regarding the serviceability of the erythrocyte staining method in capillary determinations. According to Campbell and de Langen, its weakness lies in the fact that some parts of the capillaries may only contain plasma or some of the capillaries may be open only sufficiently to permit plasma to pass through. Roberts & Wearn qualify their opinion by stating that this staining brings out only those capillaries in which there are erythrocytes at the sectioned levels. In their experience it is extremely rare that myocardial capillaries are evenly filled with blood, whereas long segments without a single red cell may frequently be encountered. When the specimen is sectioned at such a level the capillary is not visible in erythrocyte staining. Linzbach, again, has drawn attention to the irregular

distribution of capillaries in specimens stained by this method as compared to those prepared by dye injection. In his opinion this is ascribable to the fact that all the capillaries were not patent when stained by the erythrocyte staining method. He therefore considered the results open to question.

In view of these criticisms there is reason to pay particular attention to factors which influence the blood content of capillaries. Among the factors which may enter into the present experiments are anaesthesia, operative procedures and the histamine used in killing the guinea pigs. The effect of these factors has been studied previously by several workers. Thus Krogh (1922) has demonstrated that most anaesthetics, among others ether, have the effect of dilating the capillaries of the frog. The same result was reached in animal experiments (mouse, guinea pig, rabbit) by Hartman *et al.* (1928), Gianotti & Vannotti (1932), Lindgren (1935), Sjöstrand (1935) and Engstrand (1942). The last mentioned investigator studied the myocardial capillaries in particular. Sjöstrand (1935), in studying the influence of operative measures, found that the peripheral blood volume in the muscles of the guinea pig and mouse is markedly increased by laparotomy. In the opinion of several workers (*e.g.*, Dale & Richards 1918, Dale & Laidlaw 1919, Krogh 1922, Hartman *et al.* 1928, Lindgren 1935, Vannotti & Gukelberg 1935 and Sjöstrand 1935) histamine produces capillary dilatation. However, according to Dale & Laidlaw, it does not have this effect in the case of herbivorous animals, but this opinion is not supported by Lindgren or Vannotti & Gukelberg. A further observation by Dale & Laidlaw was that anaesthesia considerably increases the paralysing effect of histamine on the capillaries.

The significance of the blood volume as a factor increasing the amount of blood in myocardial capillaries in histamine-induced shock was referred to on page 25. No increase in the blood content of the capillaries was seen in the present experiments by the freezing method.

Thus, according to the above mentioned workers, anaesthesia, operative measures and histamine have an effect of a similar nature, *i.e.*, they increase the capillary blood volume, which is an advantage from the point of the present experiments. Full unanimity does not exist, it is true, regarding the effect of histamine, but at least it has not been found to decrease the capillary blood circulation.

As mentioned on page 23, considerable changes may occur in the blood content of the capillaries during the terminal stage of heart function, and it was observed that the effect of this important source of error could probably be eliminated for the greater part by the freezing method.

SOURCES OF ERROR IN THE HAEMATOXYLIN-EOSIN STAINING METHOD

Quantitative determinations of capillaries based on the staining of only the endothelial nuclei have been carried out by Thörner (1935) and Linzbach (1947). In the opinion of Linzbach, the nuclei of capillary endothelia are incontestably demonstrable and the inclusion of connective tissue nuclei is of no practical importance, since the heart muscle is very poor in connective tissue. However, von Boros (1948) believed that connective tissue proliferation in cardiac hypertrophy would detract from the serviceability of this method of determination.

The combination of nuclear staining and the staining of erythrocytes as one of the methods for quantitative capillary determinations appeared advisable, as the changes possibly occurring in the capillary blood content and the consequent source of error would not then be able to influence the results to any great extent, since the capillaries could also be counted by their epithelium and its nuclei. The possibility exists that also lymph capillaries were included, which according to Bock (1905) are present in the heart muscle in larger numbers than blood capillaries. However, if the studies by Aagaard (1924) are used as criterion, Bock's results are unreliable and the number of lymph capillaries in heart muscle is actually much smaller than that of blood capillaries.

SOURCES OF ERROR IN THE INDIA INK INJECTION METHOD

Very few criticisms have been made in the literature against the India ink injection method or against dye injection methods in general, and where made they have not usually been concerned with studies of blood capillaries of the heart. Reference should be made, however, to quantitative studies of blood capillaries of the brain by Drummond (1944), in which comparisons between the results of erythrocyte staining and India ink injection showed the two methods to be of equal value in capillary studies of the brain.

As was observed by Wearn (1928) in developing the dye injection method, the slow arrest of heart function prevents satisfactory dyeing of the capillaries. This drawback may, according to him, be corrected by stopping the heart rapidly, for example by injection of glacial acetic acid or formalin. In the present investigation, arrest of the heart function immediately after the injection of India ink was brought about by the injection of glacial acetic acid.

In the first perfusion experiments in the present investigation the heart sometimes contracted poorly or failed to contract at all. When dye was injected into a poorly beating heart the capillaries were unevenly filled with dye. Since it appeared probable that this was due to air entering the coronary arteries, the utmost care was taken to prevent this during dissection of the heart and insertion of the cannula into the aorta. The apparatus was also controlled for air bubbles before perfusion was started. Through observance of these precautions, the drawbacks mentioned were eliminated and the capillaries in the specimens were found to be evenly filled with the dye.

Since in this method the capillaries are visualised by the particles of India ink, an excessively large particle size or flocculation of the India ink on mixing with the perfusion fluid may give rise to erroneous observations. In this investigation, however, India ink of the finest particle size available was employed, the particles being mostly below 0.5 μ and the largest particles less than 2 μ in size. It is therefore to be presumed that the dye was able to penetrate into all patent capillaries. This opinion is also supported by the finding made during examination of the capillaries injected with India ink that the capillary diameter was definitely greater in all cases than the diameter of even the largest particles of India ink contained in them. In microscopic examination the India ink particles appeared to be of about the size stated by the manufacturer, and no flocculation occurred when the dye was mixed on an object glass with perfusion fluid.

An incomplete removal of the blood cells during perfusion may also be a probable source of error. As a control, a number of specimens injected with India ink were stained for erythrocytes. Isolated erythrocytes were occasionally visible in the capillaries, which usually contained also particles of India ink.

It is difficult to determine the extent to which the glacial acetic

acid employed to stop the heart action had an effect on the capillaries by washing away India ink, for the nuclear staining attempted in order to control how completely the capillaries were demonstrated by the India ink was not successful. The fact that the capillary concentrations in the specimens injected with this dye were generally somewhat lower in the present study than those obtained by a method of the same type by other workers (Shipley *et al.* 1937, Roberts & Wearn 1941, and Rotta 1943) will be discussed on page 71.

SOURCES OF ERROR IN THE HISTOLOGICAL TECHNIQUE AND EXAMINATION OF SPECIMENS

In the histological technique the possible sources of error include shrinkage of the tissues, variations in the thickness of the slices, and unsatisfactory staining of the specimens. In addition, certain other possibilities of error may be inherent in the histological treatment of the freezing method.

Pickworth (1934) stated that the shrinkage of tissues during fixation may cause emptying of the blood capillaries during the first stage of the procedure. As a result the surface of the specimen, which first undergoes fixation, may upon shrinking cause changes in the dye content of the deeper layers of tissue. This factor probably is not present in the freezing method, in which the tissues undergo fixation as defreezing progresses. It apparently is also without appreciable significance in the India ink injection method, judging by the even concentration of capillaries in the different muscle layers in the specimens.

The thickness of the slices is of significance chiefly when erythrocyte staining according to Sjöstrand is employed, for some capillaries may contain no erythrocytes at the levels at which they are cut. However, since the erythrocyte-stained slices in the present investigation were comparatively thick (15 μ) and the erythrocytes in the capillaries were usually very closely situated, it seems probable that the error due to this factor is insignificant. It might be more significant in the case of thinner slices (5 μ), but since these were stained with haematoxylin-eosin the capillary count did not depend solely on the staining of erythrocytes. This is indicated by the fact that the capillary concentration determined from these slices was usually no lower than that from only erythrocyte-stained slices cut

at 15 μ from the same tissue block. When the hearts are dyed by injection with India ink, the variations in the thickness of the slices play no part, since the entire capillary is evenly filled with dye.

There were no indications of deficient staining by either the erythrocyte or the haematoxylin-eosin staining method.

Errors arising from possible haemolysis during fixation in the freezing method was a considerable drawback not only in the counting of capillaries but also in the other determinations. Specimens showing haemolysis were therefore discarded.

Another source of error in the freezing method was the high concentration of the formalin solution used for fixation. Because of its effect on the finer details of the tissues it caused some difficulty in many cases in making determinations, especially of muscle fibres, from specimens stained with haematoxylin-eosin. This source of error was not present when the capillaries were counted in the erythrocyte-stained specimens.

Among factors which during the examination of the histological specimens may influence the results should be mentioned the choice of observation fields in the specimens. The number of observations made and possible errors in measurement may also play a part in this respect.

As reported by Wearn (1928), the capillary concentration is somewhat variable in the different regions of the heart. Therefore, in order to reach as highly comparable values as possible, all counting operations in the present investigation were made from an identical site in the hearts. Erroneous results arise if the muscle fibres in the observation field are not cut in perfect cross section, lower capillary concentrations being obtained in counts made from areas cut in oblique section than in transverse section. As mentioned by Lindgren (1935), this error increases rapidly with increase in the obliqueness of the section. To eliminate this error, the counts were only made from fields in which no deviation from the vertical direction was observed when the course of the capillaries and muscle fibres was followed at different levels of the slice. The visualisation of muscle fibrils as small dots was a further aid in determining the perfect cross section of the cut.

A sufficient number of observations is important in order to obtain reliable results. If there are marked variations in the results, a greater number of observations must be made before a reliable

value representative of the whole heart can be obtained. Since the different methods used in this investigation gave relatively uniform figures for the capillary concentration, the dispersion of the mean capillary concentration obtained with the number of observations used in this investigation was in all cases less than 5 per cent of the mean value. This speaks for the reliability of the mean values obtained.

To eliminate errors in measurement, the same microscope was employed in all the histological examinations, with the exception of those performed by the projection method. In the latter case the magnification was always controlled before examination of the specimens by comparing the projected image with a prototype.

OTHER INFLUENCING FACTORS

In addition to the above mentioned possible error factors inherent in the various methods used in this investigation, there are certain factors which influence chiefly the absolute numerical values obtained in the observations. They therefore acquire significance mainly when comparisons are made between results obtained by the different methods. Such factors are, for example, shrinkage of tissues, state of contraction of the heart, and changes in blood volume. Subjective circumstances referable to the observer may also have an influence on the results.

Tissue shrinkage due to the preparation of the histological specimens has a considerable effect on the capillary and fibre concentrations and fibre measurements, in addition to disturbing the counting operation by producing clefts in the specimens. This is true especially of the after-treatment required in the freezing method, which causes considerable shrinkage of the tissues. Treatment of the India ink-injected specimens by the gelatin method caused but slight shrinkage, as is also reported by Shipley *et al.* (1937). The values obtained from these specimens are therefore best suited for direct comparison with results obtained by other workers.

To elucidate the changes in the capillary concentration due to shrinkage of tissues, the possibility of using aqueous solutions for erythrocyte staining was tested. Although the benzidine and orthotolidin employed by Sjöstrand are poorly soluble in water, erythro-

cyte staining by an aqueous solution gave also very satisfactory results, but it was more time-consuming than when alcoholic solutions were employed. For this test the heart specimen was cut into two parts and the parts were fixed in formalin solutions of 1: 10 and 1: 5, respectively. From the first mentioned half, which was treated according to the method of Heringa & ten Berge, slices were cut with a freezing microtome, after which the erythrocytes were stained directly in an aqueous solution of the stain. While still wet the slice was placed on the object glass for calculation of the capillary concentration. The other half of the specimen was treated according to Sjöstrand's method. By the method first described, capillary concentrations of, on an average, 2,550 per square millimetre were obtained, whereas the latter method gave an average concentration of about 3,800 per square millimetre. Calculated on the basis of these capillary concentrations the tissue shrinkage in Sjöstrand's method is about 49 per cent. Actually the shrinkage is slightly greater when we consider that some shrinkage occurs also in the method of Heringa & ten Berge. According to Shipley *et al.* (1937), tissue shrinkage may vary from 2 to 3 per cent when a technique of the latter type is employed. It is thus seen that shrinkage had a marked influence on the results. The capillary concentration in the specimens treated according to Heringa & ten Berge was on a level with the results obtained from hearts dyed by India ink injection and thus is evidence supporting the opinion that the values obtained with the aid of erythrocyte staining and India ink dyeing by the methods used in this investigation correspond to each other when allowance is made for the difference caused by shrinkage.

The capillary concentration value varies according to the degree of contraction of the heart muscle, being greater in a heart stopped at the diastole than at the systole, as during diastole the muscle fibres are finer than during systole and the capillaries therefore lie closer to one another. For this reason it is difficult to compare the values obtained by different methods, since it is possible that they vary according to the degree of contraction at which the heart ceased beating. For the same reason, considerable variations may be seen in the capillary concentrations of different hearts even when only one method is used. To eliminate this source of error, the amount of blood present in the ventricles was determined in hearts

stopped by freezing. Hearts which were found to definitely differ from the other hearts in respect to the degree of contraction were discarded. Hearts dyed by the India ink injection method had without exception stopped at the beginning of the systole, and this source of error was therefore of hardly any significance in this group.

The results may also be influenced by variations in the amount of intracardiac blood. Kjellberg *et al.* (1949 a, b) observed that the blood volume tends to increase greatly during training and this in turn increases the heart volume, and Sjöstrand (1953) pointed to the effect of this increase in heart volume as a factor which, by stretching the heart walls, reduces their thickness. Thus the capillary concentrations determined from the hearts of trained animals may vary according to the rise in the intracardiac blood volume, *i.e.*, according to the elongation of the myocardial muscle fibres. However, Shipley *et al.* (1937) were unable to find any appreciable change ascribable to a cause of this kind in the capillary concentrations or fibre dimensions obtained by them. As a factor affecting the concentration of capillaries there should also be taken into consideration the possibility suggested by Sjöstrand (1953) that a trained heart with a greater volume may stop in a more contracted condition than an untrained heart.

The subjective influence of the person performing the count may be assumed to affect the results because of the intuitive selection of the counting field according to his expectation of high or low values (Shipley *et al.* 1937). However, as the mean capillary concentration in the hearts was calculated from numerous observations and the counting field was relatively large, especially in the case of specimens in erythrocyte staining or India ink injection, and as furthermore the capillaries were found to be evenly demonstrated in the specimens, it appears probable that errors due to this factor are not significant in the present investigation.

Variations may also occur in the results depending upon which blood vessels are considered by the observer to be capillaries, and upon the inclusion or exclusion of capillaries along the margin of the microscopic field. For these reasons, only those blood vessels were included in the capillary count which did not differ from the uniform size of capillaries in the specimens. In counting the marginal capillaries, only every other capillary was counted.

In addition to the possible sources of error discussed above, there also may be others which are unknown to the writer. It is to be presumed, however, that their effect, as well as that of the above mentioned factors, is counterbalanced by the fact that in all the cases in which the same method was used the procedure followed was as identical as possible.

RESULTS: GROUP I

ADAPTABILITY OF THE EXPERIMENTAL ANIMALS TO TRAINING

All the guinea pigs became well accustomed to running. Only during the last month of the training period did a few animals show definite signs of fatigue, especially during the evening training. This, however, did not prevent them from participating in the experiment to the end. Four guinea pigs were definitely better, untiring and more even runners than their comrades. Even among these four, one animal (No. 3) was outstanding. Most of the guinea pigs had a poorer capacity for running than the above mentioned four and differed very little from one another in this respect. They were classified as having an average running capacity. Three animals comprised a group of poor runners, which ran unevenly and were quickly fatigued; from time to time it was necessary to reduce their training time to one-half.

Taking these variations into consideration, the running capacity of each guinea pig is classified as good, average or poor in tables 4 and 5 (page 47 and 54). The data on the animals in these tables are listed in the order of their running capacity.

BODY WEIGHT

Table 4 shows the body weights of the animals before and after the training period. Before training the animals were of uniform weight. During the experiment the body weights of the individual control animals increased on an average by 367 ± 7 grammes, while the trained animals increased in weight by an average of 338 ± 10 grammes. The difference of -29 ± 12 between these

means is statistically almost significant ($t = 2.41$). No noteworthy difference was seen in the weight increase of the individual trained animals irrespective of their running capacities.

HEART RATE

Exact determination of the heart rate by auscultation was difficult in the case of young guinea pigs because of their rapid heart action. Electrocardiographic determination could not be used as an aid, since ether anaesthetisation would have influenced the rate. A further disturbing factor was the timidity of the control animals as compared to the trained animals, which were better accustomed to handling in connection with their daily training.

There average heart rate of the 3 weeks old guinea pigs was about 300 per minute. The rate decreased with age, being prior to killing an average of 215 per minute for the control animals. The trained animals showed signs of bradycardia already after two months' training, the heart rate averaging 182 per minute at the end of the training period. No definite correlation could be established between the heart rate and the running capacity of the animals.

No pathological changes were revealed in the trained or control guinea pigs by electrocardiograms made before the beginning of training and after its completion.

MACROSCOPIC EXAMINATION

On macroscopic examination when the thorax of the animals was opened under anaesthesia, the hearts of the trained guinea pigs generally appeared larger in size than those of the controls. The amount of fatty tissue was definitely smaller in the trained animals than in the controls, not only in the subcutaneous layer but also, to a considerable degree, in the viscera, as for example in the mesenterium and around the testes, kidneys and pericardium.

HEART WEIGHT

The heart weight, as used in this report, designates the absolute weight of the heart. This weight is listed for each animal in table 4. The average for the control guinea pigs was $1{,}904 \pm 40$ mg and for

TABLE 4

BODY WEIGHT, HEART WEIGHT, AMOUNT OF VENTRICULAR BLOOD AND RUNNING CAPACITY OF GUINEA PIGS IN GROUP I

Number of Animal	Body Weight (g)				Heart Weight (mg)		Amount of Ventricular Blood (mg)		Running Capacity
	Control Animals		Trained Animals						
	Initial	Final	Initial	Final	Control Animals	Trained Animals	Control Animals	Trained Animals	
3	160	470	195	580	1460	2290	510	1350	Good[3]
2	220	580	175	565	2020	2440	1000	1250	»
14	160	555	160	540	1880	2310	840	1430	»
18	180	510	180	590	1670	2540	810	1510	»
1	—[1]	—	190	490	—	1970	—	—[2]	Average
4	185	540	200	545	1910	2780	1230	1190	»
5	175	540	205	530	2070	2220	880	—[2]	»
6	180	540	185	500	1890	2110	540	850	»
8	210	545	190	600	1850	2580	830	1390	»
9	180	550	200	495	1780	2300	640	1440	»
10	195	535	200	580	1780	2150	1000	650	»
11	200	600	160	490	2140	2100	1260	1440	»
12	170	530	205	505	1870	2300	810	1320	»
13	—[1]	—	155	465	—	2010	—	500	»
16	185	625	165	495	2060	2200	570	720	»
17	150	535	170	460	1930	2180	890	970	»
7	150	545	175	485	2120	2300	930	760	Poor
19	155	525	170	510	1890	2110	540	670	»
20	170	530	165	435	2070	1840	720	1080	»
15	180	555	—[1]	—	1880	—	800	—	—
Mean	178	545	181	519	1904±40	2249±51	822±51	1089±82	

[1] Discarded because of sickness.
[2] Heart stopped at the systole.
[3] Best runner.

the trained guinea pigs 2,249±51 mg. The difference in the mean heart weights was +345±66 mg and shows that the increase due to training is highly significant statistically ($t = 5.25$).

Changes in the heart weight-body weight ratio due to training are shown in the graph in fig. 3. As is seen, there is a close relationship between the body and heart weights in both the control and the trained groups. This takes the form of a positive correla-

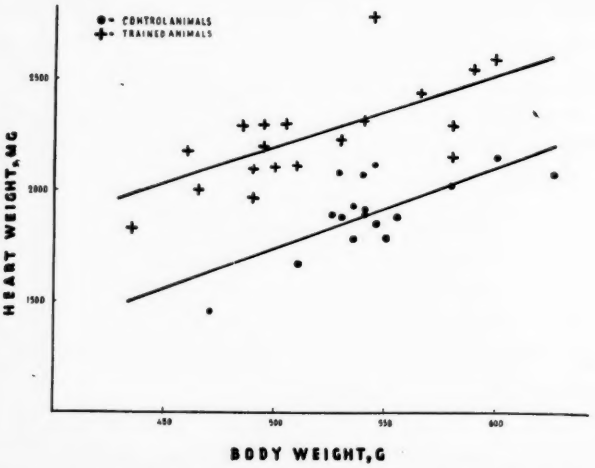

Fig. 3. — Graph showing body weights and heart weights of control and trained guinea pigs in group I. The regression lines show the dependence of the heart weights from the body weights in the two groups of animals.

tion, the coefficient (r) of which in the control animals is $+0.716$ and in the trained animals $+0.692$, and which is highly significant ($t = 4.10$) in the former and significant ($t = 3.95$) in the latter. The formula for the regression line for the control animals is as follows:

$$\text{Heart weight} = 1{,}904 + 3.628 \cdot (\text{body weight} - 545)$$

and for the trained animals as follows:

$$\text{Heart weight} = 2{,}249 + 3.277 \cdot (\text{body weight} - 519).$$

As is evident already from the nearly parallel regression lines for the control and trained animals, the heart weight of the latter ss increased with increasing body weight in approximately the iame proportion as in the control series. Thus the hypertrophy is of the same degree independently of the body weight. It is observed that the degree of myocardial hypertrophy was no greater in the best runners than in the other trained guinea pigs.

AMOUNT OF VENTRICULAR BLOOD

When the hearts were opened, attention was drawn to the greater amount of blood in the hearts of the trained guinea pigs. Determined by weight, the ventricular blood averaged 822 ± 51 mg

in the control animals and 1,089±82 mg in the trained guinea pigs. The difference of +267±95 between these values is almost significant ($t = 2.80$).

As is seen from table 4, there were considerable variations in the amount of ventricular blood in the hearts of individual control and trained animals. These variations cannot in all cases be ascribed solely to differences in the heart weights but is apparently due in these cases to differences in the degree of contraction of the ventricles.

EXTERNAL HEART MEASUREMENTS AND THICKNESS OF VENTRICULAR WALLS AND SEPTUM

On the whole, the hearts of the control guinea pigs were fairly round and those of the trained guinea pigs elongated. Measurement of the hearts after sectioning into two halves gave the following mean dimensions: Maximum length, 15.5 mm for controls and 17.2 mm for trained animals; breadth, 17.2 mm and 17.3 mm, respectively; thickness of wall of left ventricle, 2.6 mm and 2.8 mm; thickness of septum, 2.6 mm and 2.7 mm; thickness of wall of right ventricle, 1.1 mm and 1.2 mm.

CAPILLARY CONCENTRATION DEMONSTRATED BY ERYTHROCYTE STAINING

Histological Picture. — In the erythrocyte-stained specimens from control guinea pigs (fig. 4 A) the capillaries were differentiated from the nearly colourless surrounding tissue by the erythrocytes, which stained an intensive dark brown. The capillaries in the specimens were usually sufficiently large to accomodate the erythrocytes with slight or no deformation. In section the capillaries were uniformly visible over large areas and there seemed to be a capillary beside each muscle fibre. There were few areas where one capillary supplied several muscle fibres. Clefts due to shrinkage were present in many places.

The corresponding specimens from trained guinea pigs showed a very similar histological picture (fig. 4 B). The mean diameter of the capillaries in some specimens seemed slightly smaller than in the controls. In several hearts it could be seen that the capillary concentration was lower than in the controls.

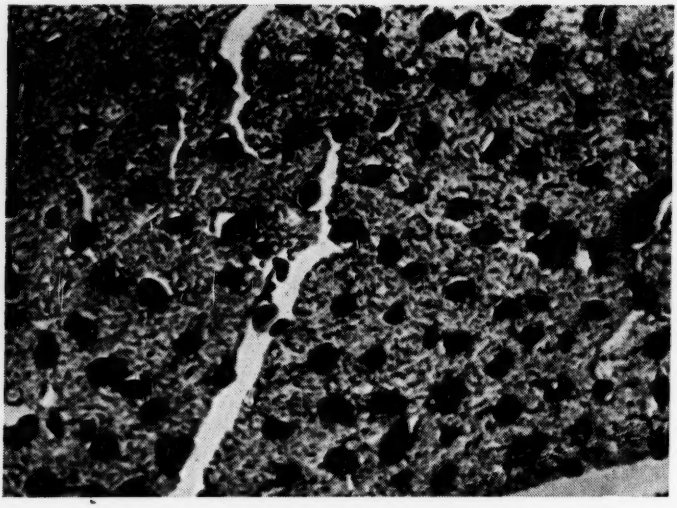

Fig. 4 A. — Photomicrograph showing the heart muscle of a control guinea pig in group I in cross section. Heart frozen with liquid air. Histological pretreatment and staining by the erythrocyte staining method of Sjöstrand. Capillaries are demonstrated by the erythrocytes, which are stained dark. Magnif. 405 ×.

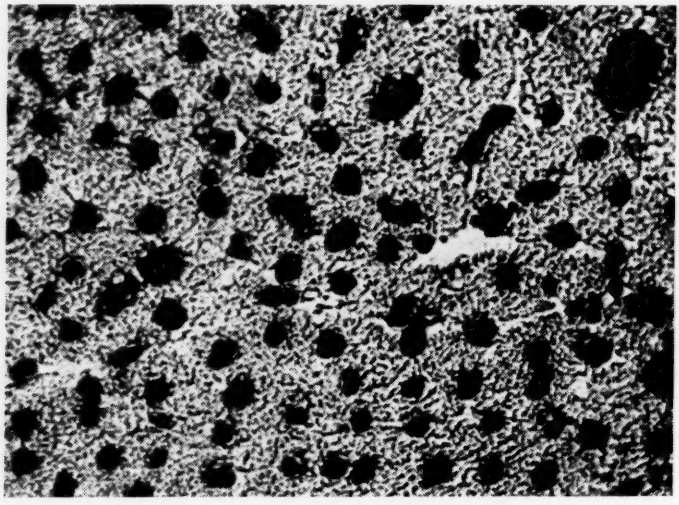

Fig. 4 B. — Corresponding specimen from a trained guinea pig. Magnif. 405 ×.

Capillary Concentration. — The capillary concentration in the heart of each guinea pig is shown in table 5 (page 54). The arithmetic mean for the group of control guinea pigs was 3,731 ± 22 and for the trained guinea pigs 3,380 ± 27. The difference of —351 ± 35 is statistically highly significant ($t = 10.03$).

In comparing the capillary concentration and the running ability of the guinea pigs it was observed that the mean capillary concentration for the best runners did not differ to any notable degree from the mean for the entire group of trained guinea pigs. Of the three highest concentrations in trained animals, two were seen in poor runners.

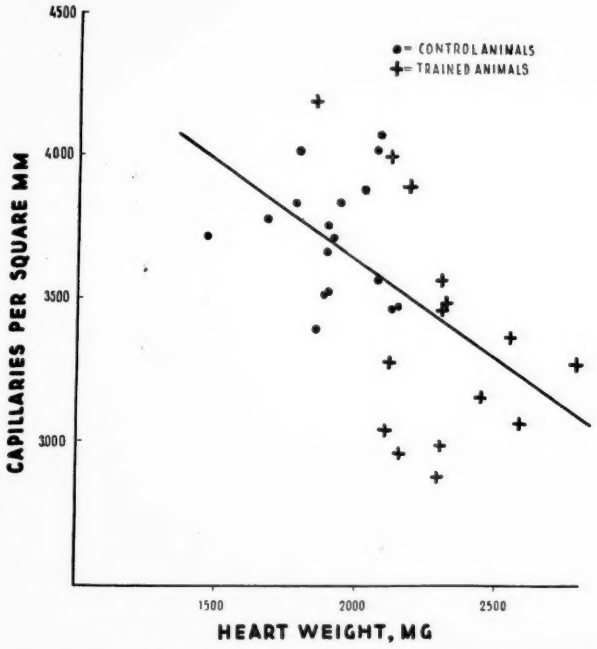

Fig. 5. — Graph showing capillary concentrations and heart weights of control and trained guinea pigs in group I, in Sjöstrand's erythrocyte staining. The regression line shows the dependence of the capillary concentration from the heart weight. Decreasing capillary concentrations are seen with increasing heart weights.

Correlation of Capillary Concentration to Heart Weight. — The graph in fig. 5 shows the capillary concentration and the heart weight of each guinea pig. It will be observed that there is a correlation between the capillary concentrations and the heart

weights. The coefficient of correlation ($r = -0.545$) is statistically significant ($t = 3.56$). From the regression line the capillary concentration in the heart can be calculated from the known heart weight by the following formula:

Capillary concentration = 3,566—0.6896 · (heart weight —2,085).

CAPILLARY CONCENTRATION DEMONSTRATED BY HAEMATOXYLIN-EOSIN STAINING

Histological Picture. — In these specimens the erythrocytes were stained red, the muscular tissue bluish, and the nuclei blue. In cross section the endothelial nuclei of the capillaries were clearly visible and distinct from the muscle fibres. The capillary wall was usually stained sufficiently to be differentiated from the surrounding tissue. In most specimens from control hearts the margins of the muscle fibres were not distinct enough to enable the counting of a sufficiently large number of fibres to give a reliable figure for the mean fibre concentration in the heart. Blurring also prevented the measurement of the fibre diameters in several cases. This drawback was present in the specimens from the trained guinea pigs to a minor degree only. In many cases the muscle fibres of the trained animals appeared to be hypertrophied. Fig. 6 A shows the histological picture of a control heart and fig. 6 B that of the heart of a trained animal.

Capillary Concentration. — The capillary concentrations for the individual hearts are shown in table 5. The arithmetic mean for the control guinea pigs was 3.999 ± 20 capillaries per square millimetre and that for the trained guinea pigs $3,623 \pm 25$ capillaries. The difference of -376 ± 31 is statistically highly significant ($t = 12.13$).

As was the case in the specimens stained for erythrocytes only, these specimens in haematoxylin-eosin staining also showed that the capillary concentration of the best runners did not notably differ from that of the other trained guinea pigs. The two highest capillary concentration values were found in poor runners.

Correlation of Capillary Concentration to Heart Weight. — The graph in fig. 7 shows the capillary concentrations and heart weights of the guinea pigs. Here also, as in erythrocyte staining, the capillary concentrations tends to decrease with increasing heart weight.

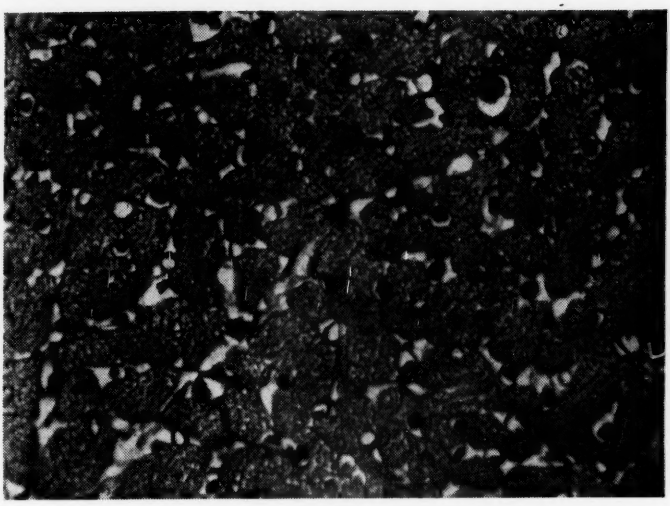

Fig. 6 A. — Photomicrograph showing the heart muscle of a control guinea pig in group I in cross section. Heart frozen with liquid air. Histological pretreatment according to Sjöstrand's erythrocyte staining method. Staining with Delafield's haematoxylin; afterstaining with eosin. Some capillaries are demonstrated by the erythrocytes, which are stained dark, and others by crescent-shaped endothelial nuclei and/or by endothelium. Magnif. 405 ×.

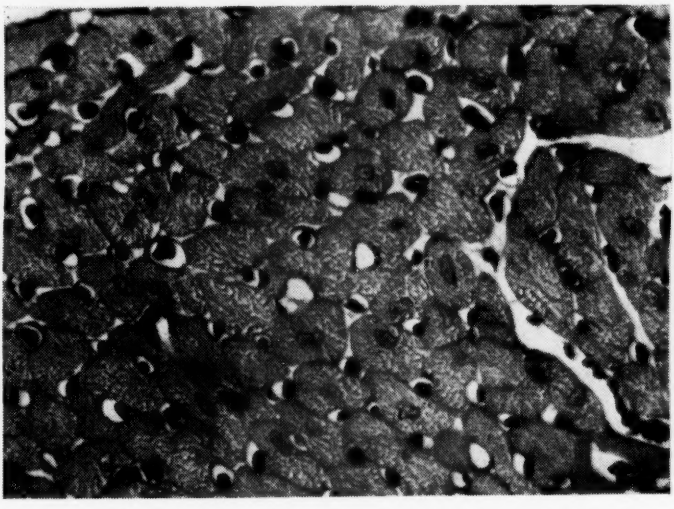

Fig. 6 B. — Corresponding specimen from a trained guinea pig. Magnif. 405 ×.

TABLE 5

MYOCARDIAL CAPILLARY CONCENTRATIONS IN ERYTHROCYTE STAINING AND HAEMATOXYLIN-EOSIN STAINING, FIBRE-CAPILLARY RATIO, FIBRE DIAMETER AND RUNNING CAPACITY OF GUINEA PIGS IN GROUP I

Number of Animal	Capillaries per sq. mm				Fibre-Capillary Ratio		Fibre Diameter (μ)		Run Cap
	Erythrocyte Staining		Haematoxylin-Eosin Staining						
	Control Animals	Trained Animals	Control Animals	Trained Animals	Control Animals	Trained Animals	Control Animals	Trained Animals	
3	3716± 62	2886± 70	3629±56	3303±66	—[4]	1.02	—[4]	14.2	Goo
2	3879± 69	3161± 91	3976±59	3461±66	—[4]	0.90	12.4	13.9	»
14	3666± 78	3490±101	3952±62	3594±66	—[4]	1.02	12.9	13.2	»
18	3775± 93	3385± 76	4529±68	3893±65	—[4]	1.22	—[4]	12.9	»
1	—[1]	—[3]	—	—	—	—	—	—	Ave
4	3719± 50	3279± 80	3794±58	3426±53	—[4]	1.11	11.8	14.2	»
5	4072±126	—[3]	4059±94	—	—[4]	—	—[4]	—	»
6	3764± 83	3288±109	3998±74	3607±71	0.97	—[4]	12.3	—[4]	»
8	3394± 99	3076± 85	3938±76	3630±56	0.91	0.94	12.1	13.6	»
9	4016± 64	3004± 75	4085±76	3660±47	—[4]	1.00	12.0	13.2	»
10	3839±119	2967± 50	3909±58	3246±54	—[4]	1.02	—[4]	14.2	»
11	3472± 87	3046±114	4134±88	3314±58	—[4]	1.08	12.9	14.9	»
12	—[2]	3570± 75	—	3428±65	—	1.24	—	14.5	»
13	—[1]	—[2]	—	—	—	—	—	—	»
16	4020± 98	—[2]	4210±94	—	—[4]	—	11.8	—	»
17	3835± 79	3894± 92	4116±84	3939±84	1.22	1.21	11.7	12.1	»
7	3466± 90	3465±112	3974±70	3338±68	—[4]	0.98	12.2	13.5	Poor
19	3526± 62	3995± 69	3774±78	4113±65	1.16	1.11	12.1	12.3	»
20	3750± 89	4187± 76	3898±54	4392±87	1.16	1.11	12.2	11.9	»
15	3521± 84	—[1]	4002±71	—	—[4]	—	—[4]	—	—
Mean	3731± 22	3380± 27	3999±20	3623±25	1.08 ±0.06	1.07 ±0.03	12.2 ±0.1	13.5 ±0.2	

[1] Discarded because of sickness.
[2] Haemolysis prevented counting.
[3] Heart stopped at the systole.
[4] Blurring of structural details in the specimen prevented counting.
[5] Best runner.

The coefficient of correlation (r) between the capillary concentrations and heart weights was —0.520, which is statistically significant ($t = 3.33$). The following formula is obtained for the regression line:

Capillary concentration = 3,823—0.6082 · (heart weight —2,085).

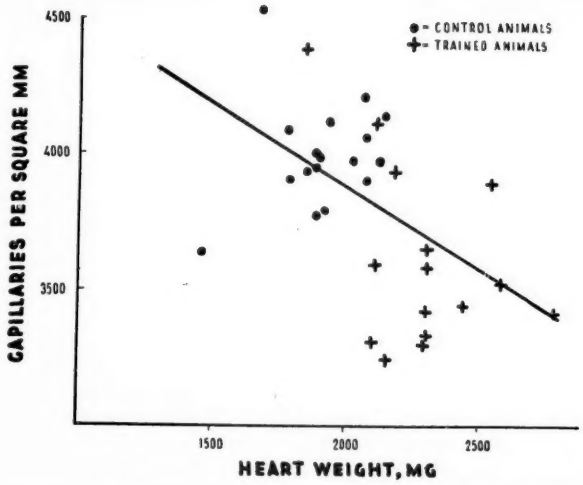

Fig. 7. — Graph showing capillary concentrations and heart weights of control and trained guinea pigs in group I, in haematoxylin-eosin staining. The regression line shows the dependence of the capillary concentration from the heart weight. Decreasing capillary concentrations are seen with increasing heart weights.

Fibre-Capillary Ratio and Fibre Diameter. — The individual ratio and measurement for each animal is listed in table 5. The arithmetic mean of the fibre-capillary ratio for normal guinea pigs was 1.08 ± 0.06 and for trained guinea pigs 1.07 ± 0.03. The difference of -0.01 ± 0.06 has no statistical significance ($t = 0.26$).

The arithmetic means of the fibre diameters were 12.2 ± 0.1 μ and 13.5 ± 0.2 μ, respectively, for the two groups. The difference in the mean fibre diameters was $+1.3 \pm 0.3$ μ, which corresponds to an increase of 11% per cent over the mean fibre diameter of the control animals. This difference is statistically highly significant ($t = 4.42$). No definite correlation was seen between the fibre diameters and the running ability of the animals.

Correlation of Fibre Area to Capillary Concentration. — The areas of cross-sectioned fibres were calculated in this investigation from the mean fibre diameter, assuming that the fibres were circular on cross section. The fibre area is used in lieu of the fibre diameter, since the capillary concentrations to which the comparison is made are expressed in relation to the unit of area. The average fibre area and the average capillary concentration in individual hearts in the groups of trained and untrained guinea pigs are

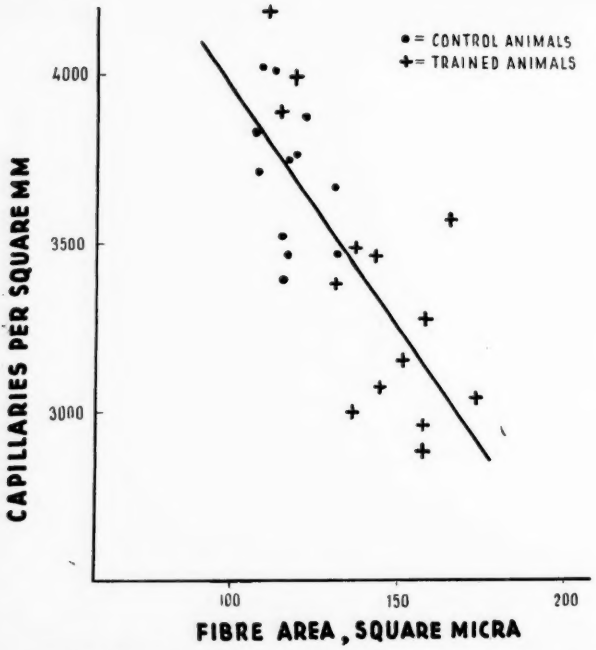

Fig. 8 A. — Graph showing capillary concentrations and fibre areas of control and trained guinea pig hearts in group I, in Sjöstrand's erythrocyte-staining. The regression line shows the dependence of the capillary concentrations from the fiber areas. Decreasing capillary concentrations are seen with increasing fibre areas.

shown in figs. 8 A and 8 B. A very close correlation is observed between the fibre areas and the capillary concentrations, the coefficients of correlation being —0.767 in erythrocyte staining and —0.868 in haematoxylin-eosin staining. Both correlations are of high statistical significance ($t = 5.85$ and 8.54, respectively). The formulas for the regression lines are as follows:

Capillary concentration = 3,535—14.12 · (fiber area —131)
Capillary concentration = 3,792—14.06 · (fiber area —131).

SUMMARY OF GROUP I

On completion of training, the mean body weight increase of the trained guinea pigs was lower than the mean increase of the controls, the difference between the means beeing statistically almost significant. During the training period the trained animals clearly developed bradycardia. No pathological changes due to training were

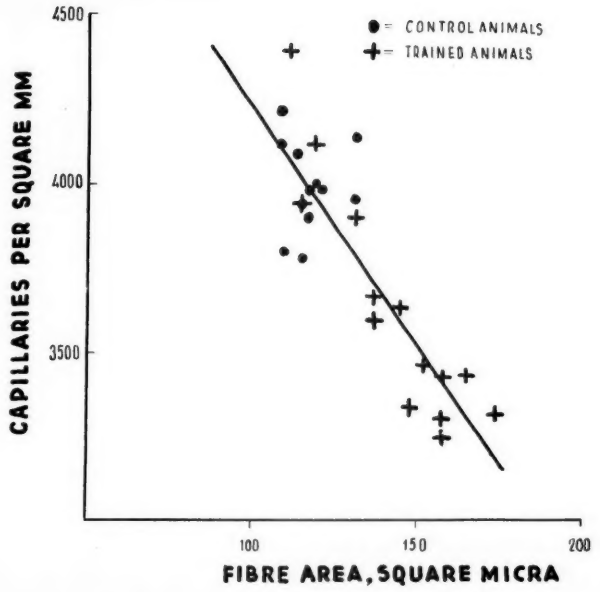

Fig. 8 B. — Graph corresponding to fig. 7 A from control and trained guinea pig hearts in group I, in haematoxylin-eosin staining. Decreased capillary concentrations are seen also here with increasing fibre areas.

revealed by electrocardiograms. Macroscopic examination of the organs showed a decrease in the amount of fatty tissue in the trained animals.

The hearts of the control guinea pigs had a mean weight of 1,904±40 mg and those of the trained animals 2,249±51 mg (hypertrophy = 18 per cent). The difference between the means was statistically highly significant. The best capacity for running was not associated with a higher degree of cardiac hypertrophy than the average for the trained animals. The hearts of the trained animals were, on the whole, elongated in comparison to the control hearts. The ventricular walls and the septum were only slightly increased in thickness. The amount of ventricular blood was in average 32 per cent greater in the hearts of the trained animals than that in the controls. This increase was statistically almost significant.

The mean capillary concentration calculated from erythrocyte-stained specimens was 3,731±22 per square millimeter for the controls and 3,380±27 per square millimeter for the trained ani-

mals. In haematoxylin-eosin staining these values were 3,999±20 and 3,623±25, respectively. The decreases in the mean capillary concentrations (351±35 in erythrocyte staining and 376±31 in haematoxylin-eosin staining) were both statistically highly significant.

In the muscle fibre-capillary ratios in the control (1.08±0.06) and trained animals (1.07±0.03) there was no statistical difference. The mean fibre diameters were 12.2±0.1 μ and 13.5±0.2 μ respectively, and the increase (11 per cent) was statistically highly significant.

The decrease in the capillary concentration was inversely proportionate to the hypertrophy of the heart muscle and the muscle fibres. The correlation between the heart weights and the capillary concentrations was significant in both erythrocyte staining and haematoxylin-eosin staining. A still more definite correlation was seen between fibre areas and the capillary concentrations, in which the correlation was highly significant in both stainings.

RESULTS: GROUP II

ADAPTABILITY OF THE EXPERIMENTAL ANIMALS TO TRAINING

As in group I, all the guinea pigs in group II became well adapted to the training experiment, with the exception that several animals began to show signs of fatigue during the last 1½ months. The first signs were seen soon after the daily training time was extended from what it was during the last 7 weeks in group I. Although the training was temporarily reduced to one-half in the case of the poorest runners as signs of fatigue increased, their running capacity became impaired once more when an attempt was made to revert to the previous training time. Three of the guinea pigs ultimately showed a complete disinclination to run and were discarded from the experiment.

Since the animals in this group were trained according to their individual running capacity, evaluation of their adaptability was easier than in group I. One of the animals (No. 20) proved superior to all others in endurance and was able to run at a time, without fatigue, for three times as long as the poorest runners. Three

guinea pigs of a fully uniform running ability ranked next, followed by two animals of good endurance. Seven guinea pigs were not so good runners as the preceding animals; they differed very little from each other. Their total amount of training exceeded slightly that of group I. Two animals were poor runners, with a running capacity comparable to that of the poor runners in group I.

The data of the guinea pigs are listed in tables 6 and 7 (pages 61 and 65) in order of theirs individual running capacity. That of the four best runners is designated as »very good», of the following two as »good», of seven as »average» and of the two poorest runners as »poor».

BODY WEIGHT

The body weights of the guinea pigs in group II are shown in table 6. Before training was started the weights of the animals to be used as controls and those to be trained were approximately equal. During the training period the control animals increased in weight from 177 g to 521 g, and the trained animals showed a smaller weight increase from 177 g to 473 g. The mean increase in the body weight of controls was 344 ± 8 grammes and of the trained animals 296 ± 9 grammes, the difference of -48 ± 12 grammes being statistically highly significant ($t = 3.88$). As in group I, the running capacity was not found to have any noteworthy influence on the increase of body weight.

HEART RATE

As was already mentioned in discussing the results for group I, the heart rate of the young 3-week-old guinea pigs was somewhat difficult to determine because of its rapidity. The mean heart rate for both the controls and the trained animals before training was 305 per minute. At the end of the experiment the heart rate of the controls had declined to approximately the level seen in group I, being on an average 224 per minute. For the group undergoing training it was slower than in the controls already at the middle of the training period, and averaged 176 per minute when training was completed. It may be mentioned that in the experiments of Frank (1950), in which the training was still more intense than in the

present group, the heart rate decreased from 240 to 160 per minute during the four months' training period.

In consideration of the greater intensity of the training in group II, a more severe bradycardia might have been expected. That this did not develop may be due to the excessive strain which the running imposed upon the animals toward the end of the training period.

No definite correlation was seen between the heart rate and the running capacity of the animals.

The electrocardiograms showed no pathological changes in either the trained guinea pigs or the controls, as was also the case in the above mentioned investigation of Frank.

MACROSCOPIC EXAMINATION

In similarity to the finding in group I, after opening of the thorax the hearts of the trained guinea pigs appeared in most cases to be clearly larger in size than those of the control animals. The amount of fatty tissue in the trained animals was smaller than in the trained animals in group I. The leg muscles of several runners appeared slightly hypertrophied as compared to the controls.

HEART WEIGHT

The heart weights of the individual animals are listed in table 6. The mean weights were $1,454 \pm 32$ mg for the controls and $1,834 \pm 57$ mg for the trained guinea pigs. The difference between the means, $+380 \pm 62$, is statistically highly significant ($t = 6.13$). As is seen from the table, the heart weights of most of the best runners were not remarkably high, the greatest heart weights being seen in the average runners.

The relationship of the heart weights to body weights is shown in fig. 9. The heart weights of the trained animals were definitely greater in relation to body weights than those of the control animals. There was a close correlation between the body and heart weights in both the control and the trained animals, seen as a statistically highly significant positive correlation ($t = 6.30$ and 4.71, respectively), with coefficients (r) of $+0.837$ for the controls and $+0.795$ for the trained animals. The regression lines for the two groups of animals are obtained from the following formulas:

TABLE 6

BODY WEIGHT, HEART WEIGHT AND RUNNING CAPACITY OF ANIMALS IN GROUP II

Number of Animal	Body Weight (g)				Heart Weight (mg)		Running Capacity
	Control Animals		Trained Animals				
	Initial	Final	Initial	Final	Control Animals	Trained Animals	
20	165	530	200	480	1520	1830	Very good[3]
3	165	525	170	530	1450	2260	»
10	160	480	170	465	1330	1890	»
16	190	505	150	440	1390	1760	»
5	160	495	220	435	1240	1880	Good
6	— [1]	—	160	470	—	1660	»
1	160	555	140	425	1500	1570	Average
4	180	460	180	455	1270	1510	»
7	170	590	190	520	1700	2130	»
9	155	515	185	485	1450	1710	»
13	195	510	150	475	1510	1830	»
14	190	565	170	435	1690	1520	»
19	190	505	185	470	1370	1960	»
8	160	540	210	530	1400	2080	Poor
18	165	520	170	480	1420	1920	»
2	180	545	— [2]	—	1650	—	—
11	190	525	— [1]	—	1410	—	—
12	200	485	— [1]	—	1300	—	—
15	215	520	— [2]	—	1380	—	—
17	180	530	— [2]	—	1650	—	—
Mean	177	521	177	473	1454±32	1834±57	

[1] Discarded because of sickness.
[2] Discarded because of disclination to run.
[3] Best runner.

$$\text{Heart weight} = 1{,}454 + 3.762 \cdot (\text{body weight} - 521)$$
$$\text{Heart weight} = 1{,}834 + 5.251 \cdot (\text{body weight} - 473).$$

As is seen from fig. 9, the rise in the regression line is slightly more abrupt for the trained animals than for the controls, which indicates that the increase in the heart weight as the body weight increases is somewhat greater in the trained animals than in the controls. In similarity with the finding in group I, the hearts of the best runners were not more hypertrophied than those of the trained animals in general.

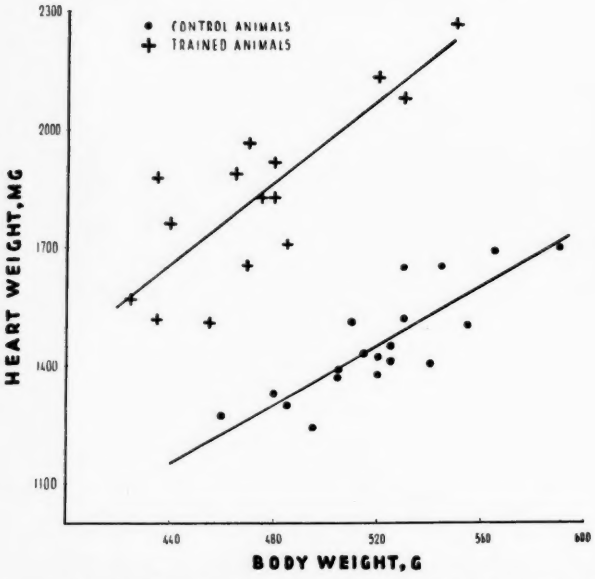

Fig. 9. — Graph showing heart weights and body weights of control and trained guinea pigs in group II. The regression lines show the dependence of the heart weights from the body weights in the two groups of animals.

EXTERNAL HEART MEASUREMENTS AND THICKNESS OF VENTRICULAR WALLS AND SEPTUM

In similarity to the finding in group I, the hearts of the trained animals generally were elongated in shape as compared to the control hearts. The latter averaged 15.2 mm and the trained hearts 17.3 mm in length. The mean heart widths were 13.8 mm for the controls and 14.9 mm for the trained animals. The mean thickness of the wall of the left ventricle in control animals was 3.3 mm, of the wall of the right ventricle 1.2 mm, and of the septum 3.0 mm. The corresponding measurements for the trained animals were 3.8 mm, 1.3 mm and 3.2 mm, respectively.

CAPILLARY CONCENTRATION DEMONSTRATED BY INDIA INK INJECTION

Histological Picture. — Contrary to the specimens prepared by the freezing method, the specimens in this group contained clefts in exceptional cases only. The capillaries were filled with India ink,

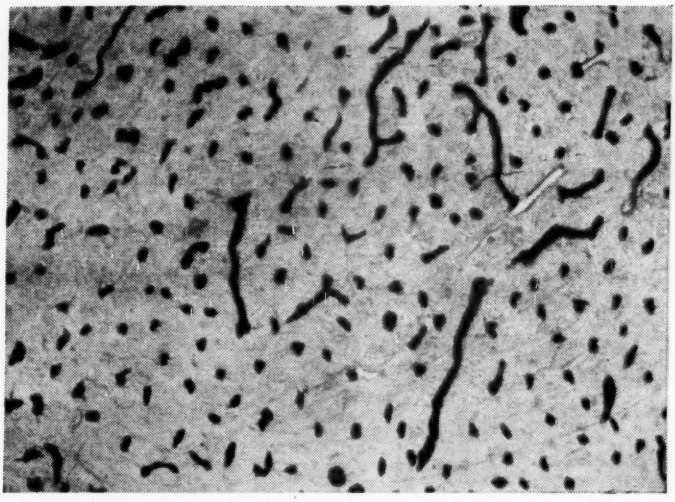

Fig. 10 A. — Photomicrograph showing the heart muscle of a control guinea pig in group II in cross section. Capillaries are demonstrated by India ink injection of the perfused heart. Histological pretreatment by the gelatin method of Heringa & ten Berge. Sections cut with ice microtome and stained lightly with eosin. Magnif. 405×.

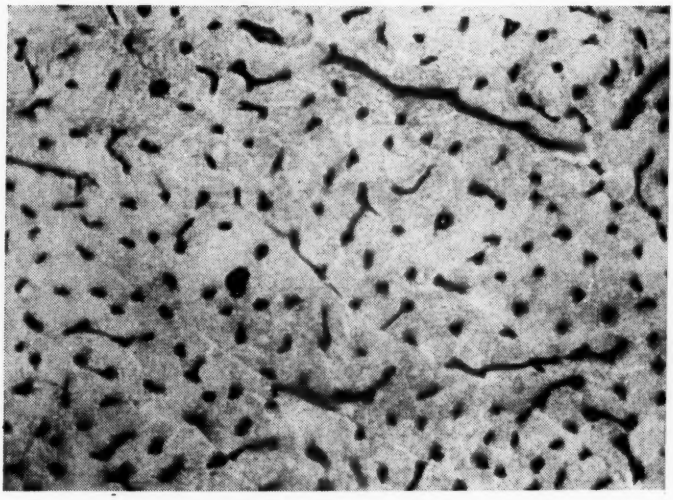

Fig. 10 B. — Corresponding specimen from a trained guinea pig. Magnif. 405 ×.

differentiating very clearly from the muscle tissue, which was stained pink with eosin. Capillary anastomoses were visible in the cross sections. There were not even minor areas devoid of capillaries, and the capillary concentration at different levels of the individual hearts was very even and of the same order of magnitude. Because of the insignificant shrinkage of the tissue, the heart muscle usually was intact and the fibre margins could not readily be distinguished. However, these specimens also contained small areas in which the fibres were more discrete, rendering counting and measurement less difficult.

No marked difference was seen between the specimens from control guinea pigs (fig. 10 A) and those from trained guinea pigs (fig. 10 B), with the exception that the capillary concentration in the latter frequently seemed to be lower and the muscle fibres thicker than in the control hearts.

Capillary Concentration. — The capillary concentrations for the guinea pigs in group II are listed in table 7. The arithmetic mean for the control animals was $2{,}641 \pm 12$ capillaries per square millimetre and for the trained animals $2{,}178 \pm 14$ per square millimetre. The decrease of 463 ± 21 is statistically highly significant ($t = 22.50$).

The lowest capillary concentration in the heart muscle was seen in the best runner. Two others among the four best runners also showed capillary concentrations below the mean concentration of the trained animals. The capillary concentrations of the two poor runners were, as also in group I, above the mean.

These numbers of animals are too small to justify the drawing of a definite conclusion concerning a relationship between the capillary concentration and the running capacity. In any case, however, a lower than normal capillary concentration had not been a hindrance to good performance.

Correlation of Capillary Concentration to Heart Weight. — In fig. 11 are shown the capillary concentrations and heart weights. The capillary concentration decreases as cardiac hypertrophy increases in the trained animals, the correlation ($r = -0.661$) between the myocardial hypertrophy and the capillary concentration being statistically highly significant ($t = 4.98$). The regression line is obtained from the following formula:

$$\text{Capillary concentration} = 2{,}437 - 0.7988 \cdot (\text{heart weight} - 1{,}622).$$

TABLE 7

MYOCARDIAL CAPILLARY CONCENTRATION, FIBRE-CAPILLARY RATIO, FIBRE DIAMETER AND RUNNING CAPACITY OF TRAINED AND CONTROL GUINEA PIGS IN GROUP II

Number of Animals	Capillaries per sq. mm		Fibre-Capillary Ratio		Fibre Diameter (μ)		Running Capacity
	Control Animals	Trained Animals	Control Animals	Trained Animals	Control Animals	Trained Animals	
20	2569±37	1763±39	1.07	1.09	16.3	21.0	Very good [3]
3	2320±42	1917±33	1.02	1.16	16.1	19.9	»
10	2734±44	2017±36	1.10	1.21	15.3	19.4	»
16	2686±28	2365±36	1.04	1.11	15.3	16.2	»
5	2662±33	1960±28	1.02	1.11	14.7	19.8	Good
6	— [1]	2172±39	—	1.14	—	17.7	»
1	2689±49	2538±34	1.05	1.26	15.4	18.1	Average
4	2553±37	1908±38	1.03	1.03	15.9	17.6	»
7	2335±36	2072±46	1.17	1.08	16.7	20.3	»
9	2709±46	2352±50	1.07	0.95	16.1	18.9	»
13	2635±23	2418±46	1.11	0.95	14.5	19.6	»
14	2729±34	2344±45	0.92	1.26	16.5	16.0	»
19	2541±52	2270±42	1.00	1.04	14.5	19.7	»
8	2607±40	2176±38	0.88	1.10	16.1	18.9	Poor
18	2625±32	2395±36	0.93	1.14	15.1	18.0	»
2	2351±47	— [2]	1.25	—	16.7	—	—
11	2547±29	— [1]	1.08	—	16.8	—	—
12	2750±38	— [1]	0.95	—	16.7	—	—
15	3145±44	— [2]	1.02	— [2]	14.6	—	—
17	2999±36	— [2]	1.22	— [2]	14.7	—	—
Mean	2641±12	2178±14	1.05±0.02	1.11±0.02	15.7±0.2	18.7±0.4	

[1] Discarded because of sickness.
[2] Discarded because of disinclination to run.
[3] Best runner.

Fibre-Capillary Ratio and Fibre Diameter. — The average fibre capillary ratio and diameter of muscle fibres in the individual hearts are listed in table 7. The group means for the fibre-capillary ratio were 1.05±0.02 for the control animals and 1.11±0.02 for the trained animals, and the mean fibre diameters 15.7±0.2 μ for the controls and 18.7±0.4 μ for the trained guinea pigs. As is seen, the largest muscle fibre diameter was present in the heart of the best runner in the group.

In similarity to group I, this group also showed no difference of any significance in the fibre-capillary ratio in the control and

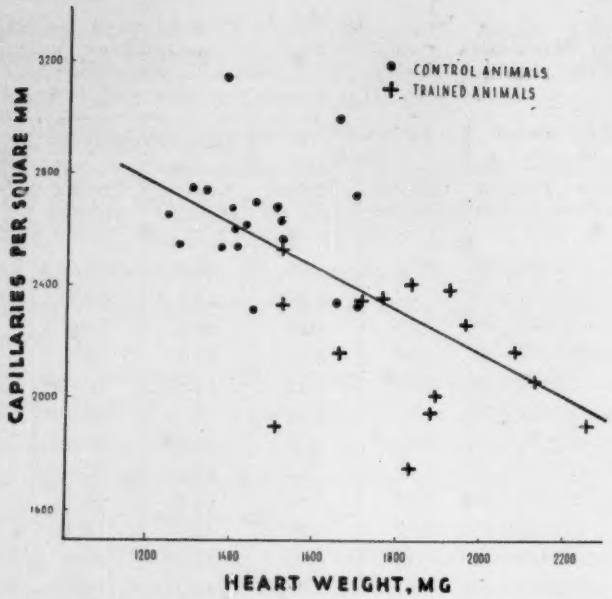

Fig. 11. — Graph showing capillary concentrations and heart weights in group II, in India ink injection. The regression line shows decreasing capillary concentrations with increasing heart weights.

trained animals (difference $+0.06 \pm 0.03$, $t = 1.82$). For fibre diameters the difference of $+3.0 \pm 0.4\ \mu$ was statistically highly significant ($t = 7.74$).

Correlation of Fibre Area to Capillary Concentration. — The fibre areas and capillary concentrations are shown in fig. 12. A decrease in the capillary concentration is seen with increasing fibre areas. The coefficient of the correlation between the fibre area and capillary concentration is —0.798, which is statistically highly significant ($t = 7.50$). The regression line is obtained from the following formula:

$$\text{Capillary concentration} = 2{,}437 - 4.807 \cdot (\text{fibre area} - 231).$$

SUMMARY OF GROUP II

The body weight of the guinea pigs undergoing training increased more slowly than that of the control animals. The difference between the mean increases in group II was more definite than in group I and was statistically highly significant. Three animals

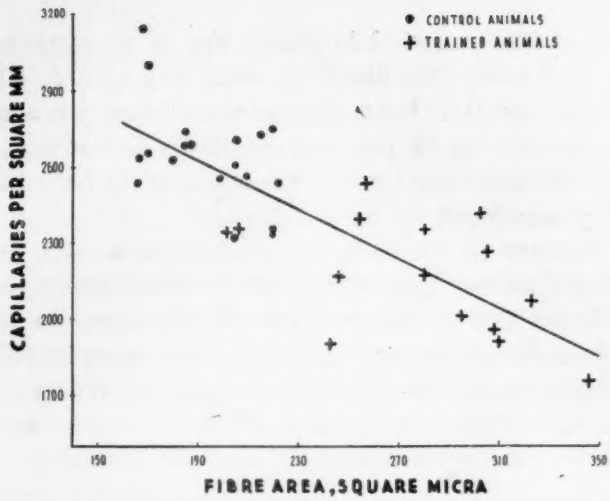

Fig. 12. — Graph showing capillary concentrations and fibre areas in group II, in India ink injection. Decreasing capillary concentrations are seen with increasing fibre areas.

had to be discarded during the strenuous training because of poor endurance. All of the animals undergoing training developed bradycardia. No pathological changes due to the training were seen in the electrocardiograms. In macroscopic examination of the organs it was found that the fatty tissue was greatly decreased in the trained guinea pigs.

The hearts of the control guinea pigs had a mean weight of $1,454 \pm 32$ mg and those of the trained animals $1,834 \pm 57$ mg (hypertrophy = 26 per cent). The difference between the mean heart weights was statistically highly significant. In similarity with the finding in group I, the best capacity for running was not associated with a higher degree of cardiac hypertrophy than the average for the trained animals. The hearts of the trained animals were elongated, and the ventricular walls (especially that of the left ventricle) and the septum were increased in thickness.

In group II (hearts injected with India ink) the control guinea pigs had a mean capillary concentration of $2,641 \pm 12$ and the trained animals $2,178 \pm 14$ capillaries per square millimetre. The decrease of 463 ± 21 (= 18 per cent) was statistically highly significant.

The difference between the average fibre-capillary ratios (con-

trols: 1.05±0.02; trained: 1.11±0.02) was of no statistical significance. The mean fibre diameters were 15.7±0.2 for the controls and 18.7±0.4 for the trained animals, which was equal to a fibre hypertrophy of 19 per cent calculated on the basis of the diameters. The difference between the means of the fibre diameters was highly significant.

The decrease in the capillary concentration was inversely proportionate to the hypertrophy of the heart muscle and the muscle fibres. The correlations between the heart weights and capillary concentrations and between the fibre areas and capillary concentrations were both statistically highly significant.

DISCUSSION

In the training experiments described in this report it was observed that young guinea pigs gradually became adapted to the increasing strain of the training. At the same time, the bradycardia typically associated with training developed. The gain in weight was slower in the trained guinea pigs than in the controls, which probably may be attributed chiefly to the smaller amount of fatty tissue in the trained animals.

Training was not observed to cause any electrocardiographic changes that could be interpreted as pathological. Most of the trained animals had developed cardiac hypertrophy of a mild degree. These hearts were hypertrophied, on an average, by 18 per cent in group I, and by 26 per cent in group II, in which the strain was more intensive.

Three different methods were employed in the examination of the myocardial capillaries. Two of these methods, based on the staining of erythrocytes according to Sjöstrand and on Delafield's haematoxylin-eosin staining, were used parallel in the same series of hearts (group I). In the other group of hearts (group II) the capillaries were demonstrated by the injection of India ink.

Using the erythrocyte staining and the haematoxylin-eosin staining, mean capillary concentrations of nearly the same level were obtained for the non-hypertrophied control hearts. The concentrations were $3{,}731 \pm 22$ capillaries per square millimetre in erythrocyte staining and $3{,}999 \pm 20$ capillaries per square millimetre in haematoxylin-eosin staining. Chiefly because of the less marked shrinkage of the tissues, the specimens dyed with India ink showed a considerably lower mean concentration of capillaries, which was $2{,}641 \pm 12$ per square millimetre. When the differences in shrinkage are taken into consideration, the capillary concentration obtained by India ink injection corresponds approximately to the value obtained in the erythrocyte-stained specimens.

All three methods of capillary demonstration showed a decrease

in the capillary concentration in hypertrophied hearts which was statistically highly significant. This decrease was 9 per cent in erythrocyte staining, 9 per cent in haematoxylin-eosin staining, and 18 per cent in India ink-injected hearts, in which cardiac hypertrophy was more marked.

The correlation between the capillary concentration and myocardial hypertrophy was statistically significant in group I and highly significant in group II. A still more definite correlation is observed between the decrease in the capillary concentration and the increase in the fibre hypertrophy, a highly significant correlation being obtained by all three methods of capillary determination. There was no change in the fibre-capillary ratio in cardiac hypertrophy which would have pointed to an increase in the number of capillaries.

Since the significance of the results greatly depends upon how completely the capillaries have been demonstrated in the specimens, comparisons are made below between capillary concentration values calculated by earlier workers and those obtained in the present investigation by the different methods employed. At the same time an effort has been made to evaluate possible factors which may give rise to differences between the results. This comparison is concerned with the values obtained by the different workers from healthy, non-hypertrophied hearts.

In the previous investigations in which erythrocyte staining was used for the capillary determinations and for which capillary concentrations have been reported (Petrén & Sylvén 1936, Petrén *et al.* 1936, Eriksson & Petrén 1937, and Petrén & Sylvén 1937a, b), this concentration was found to vary in guinea pigs within the narrow limits of 1920, and 1,970 capillaries per square millimetre. A considerable difference is seen when this value is compared with the mean capillary concentration of 3,731 per square millimetre obtained in erythrocyte-stained hearts in the present investigation. The finding of an irregular filling of capillaries with blood, which was made in connection with the preliminary training tests in the specimens in the usual formalin fixation and which was sought to be avoided by using the freezing technique, seems to indicate that the higher capillary concentration here obtained with erythrocyte staining is to be attributed to a more complete filling of the capillaries with blood.

As far as is known to the present writer, quantitative studies of the capillaries have not earlier been carried out simultaneously on the basis of both erythrocyte and nuclear staining. A comparison of the capillary concentration values obtained in this way with the values obtained from slices from the same tissue blocks stained only for erythrocytes shows that the first mentioned are higher. These higher capillary concentrations are probably due to the possibility of including in the count, through demonstration of the endothelium and its nuclei, also such capillaries that contain no erythrocytes. The possible inclusion of lymph capillaries may also have raised the value somewhat.

Chiefly owing to differences in the shrinkage during the histological treatment, the results obtained by the dye injection method by Wearn (1928) and Frank (1950) differ from those of Shipley *et al.* (1937), Roberts & Wearn (1941) and Rotta (1943). Regardless of difference in the type of material used (guinea pig, rabbit, man), the latter investigations showed a marked similarity in the results, the mean capillary concentrations ranging from 3,310 to 3,420 capillaries per square millimetre.

The results in group II of the present investigation are best comparable to the latter because of the similarity of the histological treatment. The mean capillary concentration obtained in the present investigation by the India ink injection method (2,641 capillaries per square millimetre) is 20—23 per cent lower than the above mentioned values.

Injection with India ink brought out the capillaries very uniformly, as seen from the insignificant variations in the capillary concentrations in different slices from the same heart. Not even small areas were found to be without injected capillaries. These circumstances, as well as the level of the fibre-capillary ratio, which was of the same order as in the above mentioned investigations (cf. table 8, page 76), are evidence supporting the opinion that the filling of capillaries with the dye was fairly complete. It therefore seems that the variances between the capillary concentrations in the investigations mentioned and that in the present study may be ascribed, among other factors, to possible differences in the degree of contraction of muscle fibres and of shrinkage of tissue, and to dissimilarities in the methods, such as differences in the materials, perfusion temperatures, dyes used for demonstration

of the capillaries, and treatment of the gelatin-embedded specimens.

The capillary concentrations determined by Wearn (1928) were comparatively high (4,089—5,734 per square millimetre), although he reported that his figures were corrected for shrinkage. According to Shipley *et al.* (1937), Wearn's capillary counts were made from fields containing the largest number of capillaries. The mean concentration of 2,447 capillaries per square millimetre reported by Frank (1950) is definitely lower than that obtained in the present investigation especially in consideration of the marked shrinkage caused by his histological treatment.

Summarising the comparison made above it is thus seen that the erythrocyte staining method of Sjöstrand as used in the present study yielded higher values for the capillary concentration than earlier investigations made by this method. This result is evidently due to the greater blood content of the capillaries when the freezing technique is used. Staining with Delafield's haematoxylin-eosin is to be considered comparable to the erythrocyte staining. To the knowledge of the writer it has not been used earlier for quantitative capillary determinations. The values obtained with the aid of India ink injection are lower than those obtained in corresponding earlier investigations. This may be due to differences in the techniques employed. The even distribution of injected capillaries, the small dispersion of the capillary concentrations, and the level of the fibre-capillary ratio, which was of the same order as those obtained by other workers, speak for the serviceability of the method as used in this investigation.

When a comparison similar to the one made above on control hearts is made of the capillary concentrations in hypertrophied hearts, we find that among the earlier investigators working with training hypertrophy, only Frank (1950), who used India ink injection into guinea pig hearts, was unable to observe any evidence of multiplication of capillaries. This is in agreement with the present writer's finding (Frank: non-hypertrophic hearts, 2,447 capillaries; hypertrophic hearts, 1,740 capillaries; present investigation: 2,641 and 2,178 per square millimetre, respectively).

Other investigators who have studied the capillary counts in hearts hypertrophied by training (Thörner 1935, with nuclear staining; Petrén *et al.* 1936 and Petrén & Sylvén 1937b, with erythrocyte

staining) have found an increase in capillary numbers in this condition. Thörner has not reported the capillary concentrations obtained by him. Petrén *et al.* and Petrén & Sylvén, who observed a mean concentration of *c.* 1,930 capillaries per square millimetre in non-hypertrophic hearts, found in cardiac hypertrophy the concentrations of 2,316 (Petrén *et al.*) and 2,961 (Petrén & Sylvén) capillaries per square millimetre. These values for hypertrophic hearts are definitely below the mean value for control guinea pigs in the present investigation. This marked variation is probably due chiefly to differences in the blood content of the capillaries, already referred to above, and, to a smaller extent, to possible differences in the preparatory treatment of the heart specimens. Fixation was carried out by them directly in formalin solution 1: 5, whereas in the present work the fixation was preceded by freezing of the hearts and was made in formalin solution 1: 1.

In experimental cardiac hypertrophy produced otherwise than by training, Vannotti (1936) reported results opposite to those here obtained. On the other hand, Shipley *et al.* (1937) and Rotta (1943) found the capillary concentration decreased in cardiac hypertrophy, as in the present investigation.

Linzbach (1947) considered Vannotti's findings in rabbit hearts in hypertension and aortic insufficiency open to question, since the heart muscle fibres were stated to be markedly hypertrophied, whereas, at the same time, the number of fibres per unit of area in cross section was practically the same. For demonstration of the capillaries Vannotti employed erythrocyte staining in aortic insufficiency and injection with Berlin blue in hypertension. He has given no data on capillary concentrations that would make numerical comparison with the present results possible.

Shipley *et al.* found a decrease in the capillary concentration in rabbit hearts in which hypertrophy was produced by aortic insufficiency, arteriovenous fistula or hypertension and which were prepared for examination by dye injection (concentration for control hearts, 3,420; for hypertrophic hearts, 2,670). This decrease (22 per cent[1]) is greater than that found in the present series (18 per cent) and is probably due to more pronounced hypertrophy in the material of Shipley *et al.* In pulmonary stenosis

[1] Percentage calculated by the present author on the basis of the data reported by the investigators.

produced in young rabbits (3 animals) they observed a 48 per cent[1] greater capillary count than would have been expected on the basis of the hypertrophy only (2,740 instead of an expected 1,850) and they therefore suspected that the capillaries were in this case increased in number. This in their opinion was readily comprehensible, since formation of new capillaries is known to occur during physiologic growth. When considering their results, however, one would expect a more marked shift in the capillary-fibre ratio towards capillary predominance than was observed by them (ratio in healthy hearts, 0.99; in pulmonary stenosis, 1.15). In the present investigation, no definite signs pointing to increase in the number of capillaries was found although the series comprised young animals.

Rotta's series of guinea pigs in which cardiac hypertrophy was produced by oxygen deficiency at high altitudes showed a decrease in the capillary concentration in cardiac hypertrophy (13 per cent[1]). In group II of the present investigation, to which Rotta's results are best comparable in view of the similarity of methods, a decrease of the same order was found. In his material, it is true, the cardiac hypertrophy, evaluated on the basis of hypertrophy of muscle fibres, was somewhat less marked than in the present study. As was the case with the studies of Shipley *et al.*, the capillary concentrations obtained also by Rotta were higher than the present values, presumably for reasons which have been presented above (page 71).

It may well be understood that very few studies have been carried out on hypertrophied human hearts and that the material studied has been variable also with regard to the etiological cause and degree of hypertrophy. Roberts & Wearn (1941) and Linzbach (1947), who have studied this question in various types of cardiac hypertrophy in man, observed a decrease in the capillary concentration in this condition and no signs pointing to an increase in the capillary count, unless we consider the three cases of Linzbach mentioned on page 14.

In the light of the above comparisons, in which the present results are compared with those of corresponding studies on non-hypertrophied and hypertrophied hearts, it appears that the demonstration of the capillaries in the present study has been

[1] Percentage calculated by the present author on the basis of the data reported by the investigators.

sufficiently complete to render the results serviceable for quantitative studies of the myocardial capillaries. The results show that during cardiac hypertrophy produced in growing animals by training the concentration of capillaries decreases as the hypertrophy increases. They offer evidence that cardiac hypertrophy due to training does not differ from other types of cardiac hypertrophy as far as capillaries are concerned.

A part of the quantitative studies on capillaries of the heart muscle included simultaneous determinations of the fibre-capillary ratio and fibre diameters. These are important when an effort is made to evaluate changes in the number of capillaries, for numerous studies in the field of pathological anatomy indicate that the number of fibres in the heart muscle does not increase during growth, after growth or during cardiac hypertrophy (*e.g.*, Zielonko 1874, Goldenberg 1886, Tangl 1889, Dehio 1898, Stadler 1907, Karsner *et al.* 1925, MacMahon 1937, Linzbach 1947, and Wendt & Hesse 1947). Only in exceptional cases, in the presence of a very pronounced cardiac hypertrophy (3 cases), has multiplication of fibres been stated to occur (Linzbach 1947).

Muscle fibre counts thus form the basis to which comparisons relating to capillaries may be made when the object is to elucidate whether the decrease in the capillary concentration is associated only with hypertrophy of muscle fibres or whether some compensating increase in the number of capillaries also occurs.

As was mentioned on page 73, fibre measurements gave Linzbach reason to doubt the results of Vannotti, who found in the fibre-capillary ratio a change towards greater capillary predominance in cardiac hypertrophy. In other investigations which have included studies on muscle fibres, the capillary concentration has been found, as in the present investigation, to decrease during hypertrophy without the occurrence of concomitant changes in the fibre-capillary ratio which would suggest multiplication of the capillaries.

The fibre-capillary ratios calculated by earlier workers and the present writer are shown in table 8. It will be observed that the values now obtained do not differ from those reported in the literature to any greater extent, with the exception of Vannotti's result.

The statistically highly significant correlations between fibre hypertrophy and decreased capillary concentration as well as the insignificant differences between the fibre-capillary ratios in

TABLE 8

DATA FROM STUDIES ON THE FIBRE-CAPILLARY RATIO IN THE HEART MUSCLE

Type of Heart	Wearn 1928	Vannotti 1936	Shipley *et al.* 1937	Roberts & Wearn 1941	Rotta 1943	Present Study
Non-hypertrophic	0.93—0.96	1.22 [1]	0.99—1.14 [1]	1.34	1.09	Group I 1.08
						» II 1.05
Hypertrophic	—	1.30—1.61 [1]	1.15—1.16 [1]	1.24	1.10	» I 1.07
						» II 1.11

[1] Ratio of capillaries to muscle fibres.

control and trained animals agree well with the findings of Shipley *et al* (1937), Roberts & Wearn (1941) and Rotta (1943). They are evidence supporting the opinion that no marked changes occur in the number of capillaries during cardiac hypertrophy, the decrease in the capillary concentration being ascribable mainly to the enlargement of the muscle fibres, which then push the capillaries farther apart.

To what extent this unfavourable behaviour of the capillaries in cardiac hypertrophy is present also during life, *i.e.*, how far these findings reflect the circulatory conditions in the functioning heart, is a question which cannot be answered on the basis of the studies in this investigation. It is possible that the organism is capable of compensating in other ways the unfavourable effects of hypertrophy on myocardial circulation. Without dealing more fully with this question, reference may be made to the investigation by Sjöstrand (1953) already discussed on page 44, in which he points to the stretching of the heart walls caused by the increased amount of intracardiac blood associated with training, as a factor compensating hypertrophy.

SUMMARY

The review of the literature gives a survey of the quantitative studies on blood capillaries which have been carried out in healthy and hypertrophic hearts. Attention is drawn to the small number of studies made on this subject and to the conflicting conclusions drawn in them.

The main object of the present investigation was to carry out blood capillary determinations by different methods from the hearts of experimental animals in order to throw additional light on the behaviour of the myocardial capillaries in cardiac hypertrophy.

Before the main investigation was started, preliminary experiments using guinea pigs were made to find suitable methods of capillary determination and to eliminate as far as possible the sources of error associated with each technique. As a result of this preliminary study, the capillaries were determined in the first group of the main experiments from guinea pig hearts stopped by freezing with liquid air. In these hearts the capillaries were counted by the parallel use of two methods, one of which was based on the staining of erythrocytes by the method devised by Sjöstrand, and the other on the staining of both erythrocytes and nuclei by Delafield's haematoxylin after-stained with eosin. In the second group of the main experiments the capillaries were determined by the injection of India ink into isolated guinea pig hearts revived by perfusion.

Training was selected as a suitable method for producing cardiac hypertrophy in the guinea pigs. At the age of 3 weeks the animals were subjected to running for 3½ months in two groups, each containing 20 animals subjected to training and 20 controls. In the first group the intensity of training was equal for all the animals, whereas in the second group it was increased individually according to the endurance of the animal.

A typical bradycardia developed in the trained animals. In both groups the trained animals increased in weight less than the controls. This difference in weight increase was statistically almost significant in the first group and highly significant in the second group. No pathological changes due to training were revealed by electrocardiograms.

In the group of animals subjected to the less severe training, the hearts were hypertrophied by an average of 18 per cent as compared with the controls. The hearts in the group of animals trained according to there individual running capacity were hypertrophied by 26 per cent. In the former group the mean capillary concentration calculated on the basis of erythrocyte staining was $3{,}731 \pm 22$ capillaries per square millimetre in the control animals; in the trained hearts it was $3{,}380 \pm 27$ capillaries per square millimeter, corresponding to a decrease of 9 per cent. Erythrocyte- and nuclear-stained specimens from the same hearts showed for the controls a mean capillary concentration of $3{,}999 \pm 20$ per square millimetre. In the trained hearts the concentration was $3{,}623 \pm 25$ capillaries per square millimeter, corresponding to a decrease of 9 per cent.

In the second group of animals the controls had a mean capillary concentration of $2{,}641 \pm 12$ per square millimeter, the value for the trained beeing $2{,}178 \pm 14$ per square millimeter or 18 per cent lower.

The decrease in capillary concentrations determined by the three methods was statistically highly significant and had statistically in the first group a significant and in the second group a highly significant correlation to the myocardial hypertrophy. A statistically highly significant correlation was obtained by all three methods of capillary determination between the decrease of capillary concentration and the hypertrophy of muscle fibers. No change was observed in the fibre-capillary ratios which could be interpreted as an increase in the number of capillaries. In the discussion the results obtained by the various methods of capillary determination were compared with the results obtained by corresponding methods of earlier investigators.

In reviewing the running capacity of the trained animals in the light of the results obtained, it was observed that a good running capacity was not associated with a higher degree of hypertrophy

than was seen in the trained animals in general. A capillary concentration below the average was not found to be a hindrance to a good running performance.

The findings in this investigation are evidence supporting the opinion that no multiplication of capillaries takes place in cardiac hypertrophy induced by training, the decreased capillary concentration in cardiac hypertrophy probably being due chiefly to the capillaries being pushed farther apart by the hypertrophied muscle fibres.

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